# ACTION OF MICRO-ORGANISMS ON FATS
# I. THE SIGNIFICANCE OF COLOR CHANGES IN DYES USED FOR THE DETECTION OF MICROBIAL ACTION ON FAT

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Relatively little is known concerning the action of micro-organisms on fats. Most of the tests now available for practical analytical work give only a general indication of the nature of the changes involved. This applies particularly to those methods where the action of micro-organisms is determined by the changes they produce in globules of fatty material suspended in an agar medium. The nile blue technique used by Turner (1), the copper sulphate method employed by Carnot and Mauban (2) and Berry (3), and the simple triglyceride and natural fat techniques developed by Hammer and his associates (4), all give evidence of some action of the bacteria on the fat with the probable production of free fatty acids. It is obvious, however, to those familiar with these methods, that the reactions they demonstrate are more complex than simple hydrolysis of the triglycerides to form free fatty acids. The term lipolytic as applied to micro-organisms, and as determined by the results of these and similar tests, is capable of wider interpretation.

As shown by Collins and Hammer (5) various lipolytic bacteria differ in their action on simple triglycerides. The fact that an organism produces free fatty acid from tripropionin or tributyrin is no indication that the same organism will hydrolyze mixed triglycerides of a natural fat, such as butter, lard, or cottonseed oil.

A factor further complicating the situation, and not taken into consideration in these tests, is that many organisms producing enzymes which hydrolyze the fats, also produce powerful oxidases. It is conceivable that acids formed during the later stages of oxidation may give with these tests a result similar to the action of a simple lipase. In most cases, however, hydrolysis and oxidation will be proceeding at the same time, producing the complex and varied reactions which are known to be taking place.

From a practical standpoint, when the "lipolytic" bacteria are further classified by their actions on fats, a step will be made towards better understanding of the relation between bacteria and many of the off flavors they produce in fatty foods.

The object of the work reported in this paper is to throw further light on the significance of color changes in certain dyes which may be used as indicators in fat emulsion agar media. Nile blue sulphate was used because of its apparent suitability as an indicator for this purpose, as well as the fact that it has been recommended by the Committee on Bacteriological Methods of the American Dairy Science Association (6). So far as is known, methylene blue has not been used to show changes in fat. Its widespread use in so many other ways and the general familiarity

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with its mechanism lends added interest to its application as an indicator of bacterial action on fat. p-Aminodimethylaniline monohydrochloride has been recommended by Jensen and Grettie (7) to be used in conjunction with oil emulsion agars for detecting the action of microorganisms on fats. They first grow the organism in an oil-agar medium without the addition of any indicator. Lipolytic bacteria are identified by a transparent or translucent zone around the colonies. This oxidase-detecting dye is then flooded over the surface of the agar and those colonies producing oxidizing enzymes are indicated by the deep rose-red or purple colors they assume. Further observation with the microscope shows that as well as the bacterial colonies the fat globules themselves take on various colors when this dye is added. In the first case, the color results from the direct action of the bacterial enzymes on the dye. In the second case, the dye shows, not the direct action of the enzyme, but the apparent changes the enzymes produce in the fat.

## NILE BLUE SULPHATE

The peculiar staining properties of nile blue sulphate and other dyes of the oxazine series were discovered by Smith and White (8) of Manchester University. At their request Thorpe (9) undertook to investigate the chemistry of the phenomenon. The conclusions of this early work are as follows: Applied to material containing neutral fat and fatty acids, these dyes colored the fat red and the fatty acids blue. In reality it is a double dye, the oxazine, and its corresponding oxazone produced from it by boiling in dilute sulphuric acid. Fats and many solvents (xylol, ether, petroleum ether, benzine, chloroform, carbon tetrachloride, and carbon disulphide) take up only the oxazone. Fatty acids take up both dyes and are colored from blue to purple, depending upon the relative amounts of blue and red substances present in the dye. An aqueous solution contains salts of both these bases, and the red oxazone can be increased *ad libitum* by boiling the solution with dilute sulphuric acid. This reaction is exhibited only by those dyes which are derivatives of phenonaphthoxazine. The production of the red compound results from the replacement of the amino- or substituted amino-group by hydroxyl and the consequent formation by intramolecular change of the corresponding phenonaphthoxazine.

In 1928 Turner (1) used nile blue sulphate as an indicator for lipolytic bacteria grown on an oil emulsion agar, and by comparison found it greatly superior to other methods. He also found the dye had properties of a pH indicator, changing from blue to cherry red at about 7.3 to 7.5. Later, Clark (10) gave "blue 10.2-13.0 rose" as the pH range of nile blue sulphate (nile blue A), and "blue 7.2-8.6 rose" as the pH range of a similar dye, nile blue chloride (nile blue 2B). This may explain the discrepancy in Turner's pH range for nile blue sulphate.

Collins and Hammer (5) studied the action of nile blue sulphate on various fats and fatty acids. They found tripropionin, tributyrin, tricaproin, tricaprylin, and triolein were all colored bright red; tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin were all colored red to a degree which decreased rapidly with the increase in melting points until very little red color was present. Butyric, caproic, caprylic, and oleic acids were colored uniformly blue; capric and lauric acids varied in the intensity of blue, while myristic, palmitic, and stearic acids absorbed very little

of the blue color. Beef tallow, butterfat, cocoanut oil, corn oil, cottonseed oil, lard, linseed oil, and olive oil were all colored uniformly bright red.

Jensen and Grettie (7) substituted a mineral oil in place of digestible fats in an agar emulsion, using nile blue sulphate as an indicator, and observed color reactions in colonies similar to those considered lipoclastic on vegetable oil or animal fat. They believe this phenomenon to be due to "the action of bacteria concentrated on the interfacial trap in the oil-water emulsion, acting on the dye in some manner, perhaps changing the oxidation-reduction potential."

In their "studies on Oxidation-Reduction" Cohen and Preisler (11) made an extensive investigation of nile blue sulphate. They found the oxazine to be an Eh indicator, and on the scale of electrode potential, to cover a range between methylene blue and indigo carmine. They made no mention of oxidation-reduction characteristics of the fat soluble oxazone. Apart from this one paper, little has been done to determine the action on nile blue sulphate of changes in the oxidation-reduction potential.

## THE PROBLEM

The problem in studying color changes in an oil emulsion agar medium stained with nile blue sulphate is not only the simple phenomenon of red stained fat becoming blue with the formation of free fatty acids. Many substances other than fatty acids are colored blue with the blue base of nile blue sulphate. In the globules the color may be restricted to small portions, or it may diffuse throughout the whole globule. Different organisms vary in the rate they change the red to blue; some become only shades of deeper red or purple; others change completely to blue, and with most of the lipolytic organisms the color entirely disappears from the globules after a few days' incubation. It must also be remembered that from a physical-chemical standpoint the problem is complex. There are two systems, the oil emulsion and the colloidal agar gel, each in intimate contact, each containing a portion and a different portion of the dye. Little, if any, work has been done to determine the action on the two dyes in nile blue sulphate of changes in the oxidation-reduction potential.

To determine some of these facts, after finding the pH range of the aqueous solution, much of this present work will consist of observing the reactions of this dye when separated into fatty and non-fatty solutions, as it is in the actual tests, and not only as an aqueous solution, as most others have considered it.

## EXPERIMENTAL

Stock solutions of n.b.s.[2] were prepared by dissolving one gram of "B.D.H. Nile Blue" in one liter of distilled water. To this 4 ml. of concentrated sulphuric acid were added and the solution boiled under a reflux condenser for one hour. Shaken with olive oil or xylol, this gave a deep blue aqueous layer and the xylol or olive oil was deep pink in transmitted light and had a brilliant orange fluorescence in reflected light.

The pH range was determined by adding the dye to a series of buffer solutions and then rechecking the pH of each solution separately on the

[2] In the remainder of the paper nile blue sulphate will be indicated by the letters n.b.s.

potentiometer. The dye concentration in the solutions was 1:20,000. Table 1 shows the results of these readings.

TABLE 1. *pH Range of n.b.s.*

| pH | Color |
|---|---|
| 7-10.1 | Uniformly blue |
| 10.3 | Lighter blue |
| 10.7 | Lilac |
| 11.4 | Pinkish mauve |
| 11.7 | Rose |

These results agree with those obtained by Clark (10) who gives the color range of nile blue A. as "blue 10.2-13.0 rose."

### *Effect of pH on the Color Absorbed from N.B.S. Solution by Olive Oil*

Very little is known about the effect of the pH of the aqueous solution on the red color absorbed from it by a fat or oil. In order to determine this a series of pH buffer solutions was made up with a range from about 2 to 12. One ml. of the stock n.b.s. solution was mixed with 10 ml. of the buffer solutions and their pH and Eh readings again checked on the potentiometer. Two ml. of olive oil were added to each tube and after a vigorous shaking these were centrifuged. Table 2 gives the results of these readings.

TABLE 2. *Color changes in olive oil added to a series of n.b.s. solutions of increasing pH values*

| Aqueous solution | | | Color of added oil | Final color of aqueous solution |
|---|---|---|---|---|
| Color | pH | Eh (volts) | | |
| blue | 2.50 | .82 | very pale pink | very pale blue |
| blue | 2.83 | .50 | very pale pink | very pale blue |
| blue | 5.17 | .45 | pale pink | blue |
| blue | 5.63 | .41 | pale pink | blue |
| blue | 6.00 | .39 | pink | blue |
| blue | 6.68 | .36 | red | blue |
| blue | 6.90 | .38 | red | blue |
| blue | 7.38 | .35 | purplish red | pale blue |
| blue | 8.60 | .32 | purplish red | very pale blue |
| purple | 10.90 | .23 | colorless* | pale blue |
| mauve | 11.65 | .22 | colorless* | very pale blue |

* Oil saponified and all traces of color gone

It would at first appear from this, that up to the point where saponification occurs, an increase in pH of an aqueous n.b.s. solution is accompanied by an increase in the oxazone extractable by oils or fats. However, this is not necessarily so. The change in color from pale pink to deep purplish red may have nothing at all to do with the amount of oxazone

present. As shown in later experiments the pink oxazone in fats acts as an oxidation-reduction indicator, and the change here from pale pink to purplish red is more closely related to the decrease in the oxidation-reduction potential than it is to the increase in pH.

### *Oxidation of Aqueous N.B.S. Solution*

The blue color in fat globules stained with n.b.s. and acted upon by lipolytic bacteria very often disappears after one or two days of incubation. Circumstances indicate that this is probably due to oxidation; consequently, experiments were carried out by oxidizing the dye in various ways.

Continued bubbling of air for five hours through several samples of n.b.s solution at room temperature brought about no color change. Small amounts of ozone, hydrogen peroxide, or exposure to bright sunshine caused decolorization. The addition of olive oil or xylol to samples before and after oxidation showed no increase in the oxazone. However, if oxidation was accompanied by some hydrolytic agent (increased acidity or temperature) there was an apparent increase in the oxazone as shown by the increase in the intensity of the pink color in the added oil or xylol.

Close examination of the solutions as they are being oxidized shows that decolorization is brought about by the precipitation of the dye in the form of very fine, dark suspended particles which settle out on standing. Continued oxidation after their first appearance increases the size of the

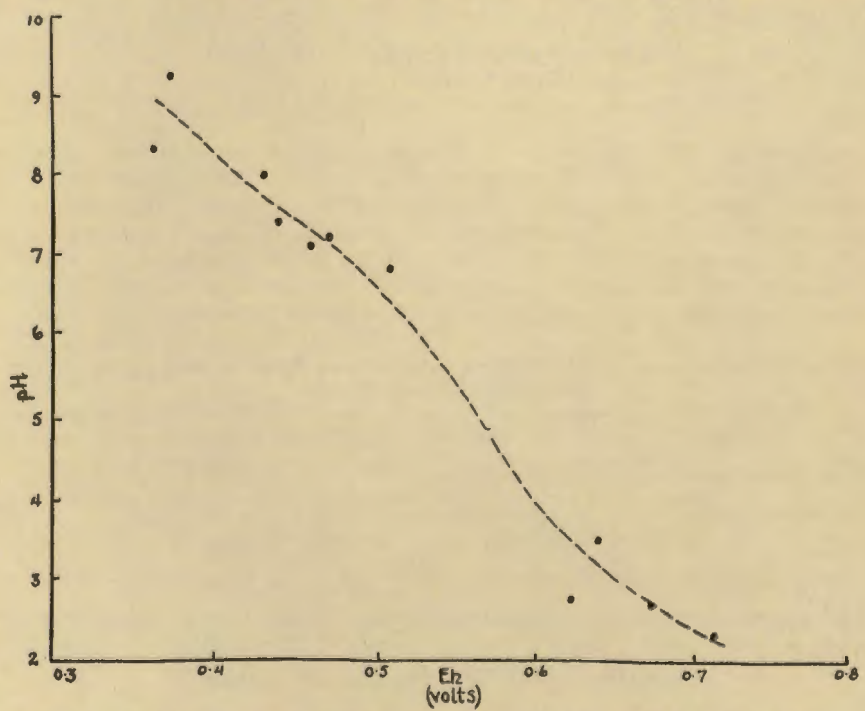


FIG. 1. Eh and pH readings at points where blue begins to fade from buffered solutions of n.b.s. being oxidized with ozone.

particles, and their color changes from blue to purple and then to red. Still further oxidation and the particles disappear entirely.

An attempt was made to find the approximate oxidation-reduction potentials, at various pH values, at which this decolorization or precipitate-formation occurs. A number of buffer solutions having a pH range from 2 to 9 were prepared. To these n.b.s. was added giving a final dye concentration of 1:20,000. A small amount of ozone was slowly bubbled through each solution until the first sign of the fine, dark precipitate appeared. A sample was removed at this point and its pH and Eh measured on the potentiometer. The data obtained are presented in figure 1. It is realized that these figures are only approximations and are not given as the exact Eh measurements at which the color change in n.b.s. occurs. The general trend, however, agrees with what is known to be true about other oxidation-reduction indicators. As the pH is increased, the Eh at which the color change takes place is decreased.

In oxidizing an aqueous solution of n.b.s. it must be remembered that there is also present some of the fat soluble oxazone. A fat extract of this pink dye is also decolorized by exposure to oxidizing agents such as ozone or hydrogen peroxide or the catalytic effect of bright sunlight. If the aqueous solution is oxidized to the point where it becomes colorless and a xylol extract is also colorless, the color change appears to be non-reversible. As an example of this, an aqueous solution was oxidized by exposure to sunlight until the color had completely disappeared. A small portion shaken with xylol left it colorless. This was then divided into three portions and treated as follows:

1. Hydrogen bubbled through it for five hours.
2. Excess sodium bisulphite added.
3. Excess sodium hydrosulphite added.

There was not the slightest color change in any of the samples. Another solution of n.b.s. was buffered at a pH value of 7 with a potassium dihydrogen phosphate-sodium hydroxide buffer mixture. This was then decolorized by slowly bubbling ozone through it. After decolorization hydrogen gas was bubbled through for three hours. As shown in table 3, in spite of the fact that Eh was reduced beyond the point where the solution had been previously colored, it remained colorless.

TABLE 3. *Potentiometer readings of an aqueous n.b.s. solution reduced by hydrogen following oxidation by ozone*

| N.b.s. solution | Color | Eh (volts) | pH |
|---|---|---|---|
| Before oxidation | deep blue | .38 | 7.0 |
| Decolorized by $O_3$ | colorless | .54 | 7.1 |
| Reduced by $H_2$ | colorless | .11 | 7.1 |

This again suggests that oxidation of the oxazine is a non-reversible reaction.

### *Oxidation of the Red, Fat Soluble Oxazone*

Oxidation of the oxazone with ozone, hydrogen peroxide and other oxidizing agents also brings about decolorization. There are some appar-

ent exceptions to this. When the oxazone is extracted in triolein or olive oil and then placed in strong sunlight, it turns to shades of yellow, green, or blue instead of becoming colorless. If it is added to xylol, or some other more stable fat solvent, and then exposed to sunshine it slowly becomes colorless. If the olive oil or triolein extract is treated with ozone or some other oxidizing agent it becomes colorless. The apparent explanation for this is that light is a catalyst and is not in itself an oxidizing agent. As well as the dye, the unsaturated fats present are easily oxidized. Where ozone is added both the oil and the dye are oxidized. Where a catalyst (sunlight) is added without the addition of an oxidixing agent, the dye becomes the donor and is reduced as the fat becomes oxidized. This is further confirmed by the fact that the addition of reducing agents to the oxazone changes it from pink or red to shades of purple, blue, or greenish blue. This is of particular interest from the standpoint of the color changes in fat globules stained with n.b.s. Under certain circumstances the catalytic action of bacterial oxidases on the unsaturated fats in the globule may bring about the reduction of the pink oxazone to shades of blue or purple. The same enzyme action on the same fat in the presence of a more available supply of oxazone may decolorize the dye instead of reducing it.

### *Addition of Reducing Agents to the Oxazone*

From the previous experiments there was an indication that the oxazone of n.b.s. may be reduced from a fat soluble pink or red to a fat soluble blue or purple form. This becomes significant when it is realized that many of the products of fat decomposition (glycerol, certain aldehydes, alcohols, and acids) are relatively strong reducing agents. In order to confirm this, many substances of various reducing capacities were added to the pink oxazone extract and their color reactions recorded.

First, a number of salts and alkalies of various reducing powers were added directly to the stained olive oil. Approximately 0.5 gram of salt was added to 5 ml. of oil. After being shaken, the tubes were left to settle and any color changes noted at the end of five minutes. At the same time about 0.5 gram of the same materials was added to approximately 5 ml. of distilled water and the pH and Eh of these aqueous solutions deter-

TABLE 4. *Color changes in the oxazone of n.b.s. in olive oil produced by the addition of various salts and alkalies, together with the pH and the approximate Eh of these same materials in a corresponding aqueous solution*

| Substance added | Color changes in stained oil | Aqueous solution pH | Aqueous solution Eh |
|---|---|---|---|
| 1. Sodium hydrosulphite | deep blue | 6.5 | −.360 |
| 2. Sodium bisulphite | blue | 6.6 | +.030 |
| 3. Sodium hydroxide | blue | 13.0 | +.043 |
| 4. Potassium hydroxide | blue | 12.6 | +.050 |
| 5. Potassium nitrite | blue | 10.2 | +.140 |
| 6. Sodium nitrite | ? | 9.0 | +.250 |
| 7. Sodium potassium tartarate | no change | 8.2 | +.290 |
| 8. Ferrous ammonium sulphate | no change | 3.7 | +.310 |
| 9. Magnesium sulphate | no change | 6.0 | +.340 |
| 10. Sodium chloride | no change | 6.6 | +.416 |

mined. In most cases the Eh was poorly poised and continued to drift slightly. The results, therefore, shown in table 4 must be regarded as simply indicating the general trend. In some of the tubes, after standing 24 hours or more the blue color disappeared.

A second series was prepared including more organic compounds. Instead of measuring electrometrically the potentials set up by aqueous solutions of these substances, their comparative reducing action was observed in an aqueous methylene blue solution. Another difference was that xylol was used in place of olive oil for the oxazone solvent. The results of these observations are seen in table 5.

TABLE 5. *Color changes in xylol solution of nile blue oxazone and in aqueous methylene blue on adding various reducing substances*

| Substance added | Color of xylol-oxazone solution | Change in methylene blue |
|---|---|---|
| Sodium hydrosulphite | bluish purple | completely reduced |
| Thymol | bluish purple | no change* |
| Phenol | purple | partially reduced |
| Betanaphthol | purple | partially reduced |
| Naphthol | purple | partially reduced |
| Indol | purple | partially reduced |
| Diphenylamine | purple | no change* |
| Amyl alcohol | mauve | reduced and extracted |
| Glacial acetic acid | mauve | slightly reduced |
| Absolute ethyl alcohol | mauve | |
| Pyrogallol | rose | slightly reduced |
| Acetone | rose | slightly reduced |
| Benzoic acid | rose | no change* |
| Chloroform | rose | no change |
| Dulcitol | pink | no change |
| Sorbitol | pink | no change |

* Insoluble or very slightly soluble in water.

Aqueous solutions of hydroquinone, glycerol, elon, and ascorbic, citric, malic, acetic, lactic, and tartaric acids all became blue when shaken with solutions of the oxazone in xylol. Concentrated glycerol, lactic, acetic, and citric acids shaken with the xylol extract also turned to various shades of blue. When olive oil was substituted for xylol as the solvent of the oxazone, the results were similar, with slight variations.

### *Fatty Acids*

Added to aqueous n.b.s. the liquid fatty acids readily absorb the dye, which remains unchanged. But added to an olive oil or xylol solution of the oxazone, the pink color is changed to shades varying from rosy pink to mauve. None of the fatty acids was able to change the oxazone from pink to blue as has so often been suggested in literature on this subject. Each fatty acid appears to produce its own peculiar color, and the addition of an excess simply increases the depth of color without changing its shade. The crystals of the higher fatty acids remain colorless in the

presence of oxazone until they are melted, when they too take on various shades of mauve.

### *Absorption of Aqueous Nile Blue into Fat or Oil from a Surrounding Agar Medium*

From the preceding experiments it seems obvious that if the pink oxazone in the globules themselves is the source of the color when bacterial enzymes turn the globules blue, the mechanism must be one of reduction. There is, however, a second method by which globules may turn blue. If, in the decomposition of the fat, a substance is formed in which blue dye is soluble, and which is also fat soluble, this substance may extract the blue from the aqueous to the fatty phase. In order to test this action the following experiment was run: Five ml. of beef extract agar were stained with n.b.s. and put into each of fifteen test tubes and allowed to harden. On top of this was placed either 1 ml. of pure fatty acid or 1 ml. of olive oil containing two drops of fatty acid or some other substance. These were corked and left to stand at room temperature for 24 hours. As shown in table 6, the lower fatty acids, either concentrated or diluted in oil, are able to extract the blue color from the adjacent stained agar. This is very marked in the case of butyric acid, which is completely soluble in water. As the acids become less soluble in water, naturally this power of absorbing the blue dye decreases. It is interesting to note that substances other than fatty acids are able to extract the color in exactly the same manner. Thymol, and amyl and isobutyl alcohols, which are soluble in olive oil, absorbed the blue dye in this way. Lactic acid, which is not fat soluble, absorbed the blue dye, but did not color the oil.

TABLE 6. *Color extracted from beef extract agar stained with n.b.s. by various substances*

| Substance added | Color extracted |
|---|---|
| Olive oil | none |
| Concentrated lactic acid | deep blue* |
| Concentrated butyric acid | deep blue |
| Concentrated caproic acid | pale blue |
| Concentrated oleic acid | pale blue |
| 10 per cent lactic acid in olive oil | acid blue; oil unchanged |
| 10 per cent butyric acid in olive oil | pale blue |
| 10 per cent caproic acid in olive oil | pale blue? |
| 10 per cent oleic acid in olive oil | very pale blue |
| 10 per cent lauric acid in olive oil | very pale blue |
| 10 per cent myristic acid in olive oil | unchanged |
| 10 per cent palmitic acid in olive oil | unchanged |
| 10 per cent thymol in olive oil | very pale blue |
| 10 per cent isobutyl alcohol in olive oil | very pale blue |
| 10 per cent amyl alcohol in olive oil | very pale blue |

* In each case where the added substance became blue there was a noticeable decolorization of the adjacent agar.

## DISCUSSION

In most of the previous work with nile blue sulphate this dye has been considered either as a pH indicator (1), a specific test for fatty acids (8), or for the unsaturated fatty acids (13). The observations reported in this paper suggest that there is some need for a revision of our explanation of its mechanism.

It must be realized that n.b.s., as used for detecting lipolytic organisms, is a double dye. The fat in the medium contains only the pink oxazone and the agar may contain both the oxazine and the oxazone. When the fat globules turn blue under the influence of microbial enzymes, the blue color may come from either one of two sources. By reduction the pink oxazone within the globule may be changed to a fat soluble blue; or the blue may be extracted from the adjacent agar medium by the formation within the globule of some substance sufficiently soluble to absorb the dye.

Eisenberg (12), in a recent article on this subject, states, "When a solution of free oxazone in oil is mixed with a fatty acid, such as oleic acid, the red color is changed to blue. This reaction is specific and forms the basis of the following medium." In the experiments reported in this paper none of the fatty acids changed the pink oxazone to blue. At best, they produced shades of mauve; oleic acid produced a distinct rose color. But many substances other than fatty acids did change the oxazone from red to purple or blue. If these substances were soluble in the solvent containing the dye the whole solution changed color. If they were not soluble in the solvent containing the oxazone, they extracted the color and at the same time reduced it to purple or blue. These substances are all reducing agents, and the amount they change the color from red through rose, mauve, purple to blue depends upon their strength as reducing agents. This probably explains the facts observed by Jensen and Grettie (7) that under some circumstances globules turn blue under the influence of bacterial enzymes, even when petroleum oil is substituted for a digestible fat.

The other source of blue in the fat globules is by the formation of some substance which is fat soluble and at the same time will readily absorb the blue oxazine. The lower fatty acids and more especially those which are water-soluble have these characteristics. Experiments demonstrating this have been described. However, this is not specific for fatty acids. Other substances such as isobutyl and amyl alcohols, which are soluble in fat, will absorb the blue from adjacent agar stained with n.b.s. Lactic acid is not soluble in fat, but when mixed with fat and brought in contact with the stained agar the acid alone extracts the blue dye. This probably explains the presence of minute, isolated, blue droplets in globules of fat which can occasionally be observed with certain non-lipolytic bacteria in fat emulsion plates stained with n.b.s.

It seems apparent then that oxidation and reduction may be more important than has heretofore been considered in using and interpreting the nile blue sulphate technique for studying changes brought about by micro-organisms in fats. The possibilities are that most organisms producing blue in fat stained with n.b.s. do so by hydrolyzing the fat, freeing fatty acids which absorb the blue aqueous dye. It is also possible that organisms unable to hydrolyze the fat may produce a blue color in the stained fat globules by means of oxidation-reduction reactions. Considering the number of complex fats and oils used with this dye, and considering the paucity of our knowledge concerning bacterial oxidases, their action on fat and the by-products produced, it seems evident that further investigation is needed before any definite conclusions can be drawn as to the exact significance of color changes in fat stained with this dye.

From a practical standpoint, however, this dye should continue to serve a useful purpse. A positive reaction means that the micro-organisms

are having some destructive action on the fat. Whether or not the finer mechanisms of the reactions can be explained will not alter the offensive flavors such organisms may produce in dairy products or other fatty foods.

## METHYLENE BLUE

When an aqueous solution of methylene blue is incorporated into an agar medium containing butter fat, it is reduced to its colorless form during autoclaving. Microscopic examination shows that the fat globules are also free from color. When these plates are inoculated with certain types of bacteria, microscopic examination shows that the fat globules adjacent to these colonies turn blue. This involves the double problem of discovering the method by which the dye is apparently transferred to the fat phase, as well as the mechanism that brings about the reversion to the colored form.

Many of the organisms bringing about this change are among those generally considered to be lipolytic. For that reason experiments were conducted to find the effect of fatty acids, and various other substances on methylene blue. One ml. of 1.5 per cent methylene blue was added to one liter of water. Sufficient sodium acid sulphate was added to decolorize the solution after five minutes boiling. This was then adjusted with sodium hydroxide to a pH of 6.75. Two ml. portions of this solution were transferred to small serological test tubes. Approximately 0.25 to 0.5 ml. of each of the substances enumerated in table 7 was added to each tube and their color changes noted over a period of 24 hours at room temperature. A similar series using the oxidized form of the dye was also prepared.

These results, shown in tables 7 and 8, indicate that olive oil, and at least those triglycerides used, do not absorb the dye from the aqueous solution. Also, that under the conditions in which they were incubated, they produced no color change on either the reduced or oxidized forms of methylene blue when the dye is associated with them in an aqueous solution. That this is not the case with unsaturated fats under conditions which favor oxidation is shown by the fact that, in several tests, change in the color of methylene blue is used as a measure of keeping quality. However, triolein, under conditions more nearly comparable to those in the agar plates incubated in the dark at room temperatures, will not reduce the oxidized form of methylene blue.

The saturated fatty acids, especially those of the lower molecular weights, readily extract methylene blue from the aqueous solution, and if it has been previously reduced, change it back to its oxidized form. Oleic acid, in common with the others, absorbs the dye; it differs from the saturated acids in slowly reducing the methylene blue to its colorless form. This is probably due to the withdrawal of oxygen from the solution as the acid itself is oxidized.

Amyl and isobutyl alcohols act towards methylene blue in a manner similar to the unsaturated fatty acids. They extract the dye from an aqueous solution and, if it is reduced, oxidize it.

Acetic, lactic, citric, and amino succinic acids, which are not fat soluble, oxidize the reduced form of the dye, but do so less completely than the lower fatty acids. Acetone (a ketone) and glycerol act as reducing agents towards methylene blue. Formaldehyde very slowly permits a pale blue color to return to the reduced form of the dye.

TABLE 7. *The oxidizing action of various substances as shown by the formation of a blue color when added to reduced methylene blue*

| Substance added | Color of aqueous phase | Color of non-aqueous phase |
|---|---|---|
| 1. Control | colorless | ..... |
| 2. Olive oil | colorless | colorless |
| 3. Tricaproin | colorless | colorless |
| 4. Tricaprylin | colorless | colorless |
| 5. Trimyristin | colorless | colorless |
| 6. Tripalmitin | colorless | colorless |
| 7. Glycerol | colorless | ..... |
| 8. Oleic acid | colorless | colorless |
| 9. Butyric acid | blue | ..... |
| 10. Caproic acid | almost colorless | blue |
| 11. Caprylic acid | almost colorless | blue |
| 12. Lauric acid | colorless | pale blue |
| 13. Myristic acid | colorless | very pale blue at surface |
| 14. 3 percent hydrogen peroxide | blue | ..... |
| 15. Sodium chloride | top half blue | ..... |
| 16. Lactose | colorless? | ..... |
| 17. Acetone | colorless | ..... |
| 18. Formaldehyde | pale blue | ..... |
| 19. Russian oil | colorless | colorless |
| 20. Hydroquinone | colorless | ..... |
| 21. Trimethylamine | colorless | ..... |
| 22. 95 per cent alcohol | pale blue at surface | ..... |
| 23. Amyl alcohol | colorless | blue |
| 24. Sorbitol | colorless | ..... |
| 25. Dulcitol | colorless | ..... |
| 26. Isobutyl alcohol | colorless | blue |
| 27. Lactic acid | top half blue | ..... |
| 28. Acetic acid | top half blue | ..... |
| 29. Citric acid | top half blue | ..... |
| 30. Uric acid | colorless | ..... |
| 31. Amino succinic acid | blue | ..... |

The controls of both the oxidized and reduced dye solutions remained unchanged throughout the duration of these experiments.

## DISCUSSION

From the standpoint of explaining the color changes in the fat globules these observations suggest several different things. The fats themselves do not absorb the dye. The color therefore must be due to some by-product of fat decomposition which will both extract the dye from its aqueous solution and at the same time oxidize it. The lower saturated fatty acids have these characteristics. It is probable that in the majority of cases where fat globules turn blue in the presence of reduced methylene blue, the production of free fatty acids is the cause. It is also possible that small amounts of other substances capable of extracting and oxidizing reduced methylene blue might be formed during decomposition of fats by bacteria. Peroxides, and some of the carboxy acids, might be the cause of

TABLE 8. *Color changes following the addition of various substances to solutions of methylene blue in its oxidized forms*

| Substance added | Aqueous phase | Non-aqueous phase |
|---|---|---|
| Control | blue | ..... |
| Olive oil | blue | colorless* |
| Triolein | blue | colorless |
| Tricaproin | blue | colorless |
| Trilaurin | blue | colorless |
| Glycerol | pale blue | ..... |
| Butyric acid | blue | ..... |
| Caproic acid | blue | blue |
| Caprylic acid | blue | blue |
| Lauric acid | blue | pale blue |
| Myristic acid | blue | colorless |
| Palmitic acid | blue | colorless |
| Oleic acid | blue | blue |
| Lactic acid | blue | ..... |

* Natural color of oil etc., not considered in these descriptions.

isolated blue droplets in the globules. As they are not fat-soluble the blue they abstract will not diffuse throughout the whole globule but remain in isolated areas.

### p-AMINODIMETHYLANILINE MONOHYDROCHLORIDE

Like methylene blue, p-aminodimethylaniline monohydrochloride is an oxidation-reduction indicator. It was originally used by Dietrich and Liebermeister (14) for demonstrating the existence of oxidizing granules in the anthrax bacillus. By incorporating this dye, together with an alkaline solution of a-naphthol, into agar, Schultze (15) produced a pale blue medium which would turn to bluish black when streaked with a heavy suspension of certain oxidizing bacteria. Gordon and McLeod (16) repeated and extended the observations of Schultze, advocating this technique as an aid in differentiating closely related organisms. They attempted to incorporate this reagent in a culture medium but found the amount of dye needed to produce the reaction would inhibit growth. Ellingworth, McLeod, and Gordon (17) investigated the similar use of other diamines and found the tetramethyl compound to be the most sensitive and satisfactory for bacteriological technique. They recommended that colonies be flooded with a 0.5 per cent solution of this dye, which gives the oxidizing colonies a deep bluish-violet color.

Jensen and Grettie (7) combined the use of this dye with oil emulsion agar media for diagnosing the action of bacteria on fats. Physical changes in the globules producing a translucent zone around the colonies, they considered to indicate hydrolysis. The reaction of one of these dyes flooded over the surface of the agar indicated whether or not the colony also produced oxidizing enzymes. In this way they were able to distinguish between those organisms which hydrolyze the fat, those which oxidize it, and those which produce both reactions.

The observations of the investigators mentioned above concerned

color changes in the aqueous solution of the dye coming in contact with the bacterial colony. Microscopic observations on the fat globules of oil-emulsion agar plates flooded with the dimethyl compound show characteristic color changes in the fat globules themselves. They may be red, yellow, brown, black, or colorless. Inasmuch as these changes are the result of the action of the bacterial enzymes on the fat itself, their interpretation may be important in the study of lipolytic organisms.

## EXPERIMENTAL

A 0.5 per cent aqueous solution of p-aminodimethylaniline monohydrochloride was prepared and one ml. portions were placed in small serological test tubes. Approximately 0.25 ml. of the various substances enumerated in table 9 were then added to the tubes. These were shaken and allowed to settle. In the case of the solid fats and fatty acids they were observed first in the crystal form and later slowly raised to their melting points. Table 9 shows the results of these observations.

TABLE 9. *Color changes after the addition of various substances to a 0.5 per cent aqueous solution of p-aminodimethylaniline monohydrochloride*

| Substance added | Color changes | |
|---|---|---|
| | Aqueous solution | Non-aqueous phase |
| Olive oil | none | none |
| Triolein | much lighter | light amber |
| Tricaproin | lighter | very light brown |
| Tricaprylin | lighter | little change |
| Trilaurin | none | little change |
| Trimyristin | none | very light brown |
| Tripalmitin | none | little change |
| Tristearin | none | little change |
| Butyric acid | paler? | none formed |
| Caproic acid | much paler | almost black |
| Caprylic acid | much paler | dark brown |
| Lauric acid | paler | almost black* |
| Myristic acid | not much change | grayish brown* |
| Palmitic acid | not much change | grayish brown* |
| Stearic acid | not much change | grayish brown* |
| Oleic acid | much lighter | light brown |
| Glycerol | paler (slowly) | none formed |
| Xylol | none | colorless |
| Lactic acid | not much change | none formed |
| Amyl alcohol | not much change | very dark purple |
| Absolute methyl alcohol | not much change | none formed |
| 30 per cent hydrogen peroxide | deeper color | none formed |
| 3 per cent hydrogen peroxide | slightly deeper | none formed |

* Only after melting.

These observations show that olive oil and at least those triglycerides used either do not absorb this dye from an aqueous solution or do so very slowly. The fatty acids, on the other hand, all absorb the dye from the

aqueous sloution. Butyric acid, which is completely soluble in water, naturally differs from the others in this point.

When this dye is absorbed from an aqueous solution by fatty acids it changes to colors varying from brown to black. The depth of the color varies roughly with the decrease in molecular weight of the acids. It is significant that where butyric acid is mixed with the dye this color change is not noted. As shown by the action of amyl alcohol, some substances other than fatty acids, which are also not water soluble, are able to extract the dye and deepen its color.

Relatively powerful oxidizing agents, such as 30 per cent hydrogen peroxide, when added to the dye solution, increase the depth of the color, and reducing agents such as glycerol reduce the color. But even strong hydrogen peroxide does not increase the depth of the color as much as when it comes in contact with the lower fatty acids that are not completely water soluble.

Similar experiments with the corresponding tetra-methyl compound did not give results similar to those described above.

## GENERAL CONCLUSIONS

1. The mechanisms underlying color changes in fat globules in a medium containing any one of the three dyes studied in this paper are not always simple reactions of the same type. Therefore such color changes cannot be used as specific indications of any one type of reaction (i. e. hydrolysis of the fat).

2. The lower fatty acids, either alone or mixed with fat, have the power of extracting these three dyes from an aqueous solution. If the dyes are in a colorless form in the aqueous solution, they are changed to their colored forms after being abstracted by the acids. Some substances other than fatty acids are able to extract the dye from the aqueous to fatty material. It is probable that most of the color changes observed in fat emulsion are due to reactions of this type.

3. In the case of nile blue sulphate, besides extraction of the blue from the aqueous solution by fatty acids or other substances within the globules, similar color changes may be brought about by the reduction of the oxazone from a pink to a blue color. Fatty acids will not reduce the oxazone from pink to blue.

## REFERENCES

1. Turner, R. H.
1929. The action of bacteria on fat. Jour. Infect. Dis., 44:126.

2. Carnot, P., and H. Mauban
1918. Réaction colorée de la stéapsin sur la plaques de gelose-graisse emulsionée par production de savon de cuivre. Compt. rend. Soc. de Biol., 81:98.

3. Berry, J. A.
1933. Detection of microbial lipase by copper soap formation. Jour. Bacteriol., 25:433.

4. Long, H. F., and B. W. Hammer
1937. Methods for the detection of lipolysis by microorganisms. Iowa State College Jour. of Science., 11:343-351.

5. COLLINS, M. A., and B. W. HAMMER
1934. The action of certain bacteria on some simple triglycerides and neutral fats, as shown by Nile Blue Sulphate. Jour. Bacteriol., 27:473.

6. COMMITTEE REPORTS
1933. Jour. Dairy Sci., 16:294.

7. JENSEN, L. B., and D. P. GRETTIE
1937. Action of micro-organisms on fats. Food Research, 2:97.

8. SMITH, L., and P. WHITE
1907. On the simultaneous staining of neutral fats and fatty acids by oxazine dyes. Jour. Path. and Bacteriol., 12:1.

9. THORPE, J. F.
1907. A reaction of certain coloring matters of the oxazine series. Transactions in the Jour. of Chemical Soc., Lond., 91:324.

10. CLARK, W. M.
1922. The determination of hydrogen ions. Williams & Wilkins Co., Baltimore.

11. COHEN, B., and P. W. PREISLER
1931. Studies on oxidation-reduction. XVI. The oxazines: nile blue, brilliant cresyl blue, methyl capri blue, and ethyl capri blue. Suppl. No. 92 to the U. S. Public Health Reports.

12. EISENBERG, G. M.
1939. A nile blue culture medium for lipolytic micro-organisms. Stain Technology, 14: (2)63.

13. KAUFMANN, C., and E. LEHMANN
1926. Virchows Arch. Path. Anat., 261, p. 623 (quoted by Turner, see reference No. 1).

14. DIETRICH, A., and G. LIEBERMEISTER
1902. Centr. f. Bakt. Orig., 32:858.

15. SCHULTZE, H.
1910. Über eine neue methode zum nachweis von reduktions-und oxydationswirkungen. Centr. f. Bakt. Abt. 1, 56:544.

16. GORDON, J., and J. W. MCLEOD
1928. The practical application of direct oxidase reaction in bacteriology. Jour. Path. and Bacteriol., 31:185.

17. ELLINGWORTH, S., J. W. MCLEOD, and J. GORDON
1929. Further observations on the oxidation by bacteria of compounds of the para-phenylene diamine series. Jour. Path. and Bacteriol., 32:173.

# A STUDY OF CHOLESTERILENE[1]

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In our investigation of the dehydration of cholesterol (1, 2) several points relevant to a better understanding of the problem were studied. If the specific rotation of cholesterilene (3,5-cholestadiene (2)) is to be considered the highest negative rotation observed (—123.23 (2)), then the samples of cholesteriline of lower negative specific rotation prepared by various methods (1) must be impure. A study of the preparation of cholesterilene by the copper sulfate method demonstrated the point that cholesterilene possessing the highest negative rotation attainable on purification was obtained from the initial crude product having the highest negative rotation. Thus the method of preparation was found to be of more importance than the subsequent purification of the product obtained in order to produce the compound of highest purity. It was also indicated that cholesterilene possessing the more negative specific rotation was obtained at the lower reaction temperatures used although the time necessary for the dehydration was increased. A decrease in the conditions favorable for pyrolytic side reactions was found to be of importance since the impurities in crude cholesterilene are difficult to remove.

Cholesterilene obtained by the zinc dust distillation of cholesterol has been reported (3) to have the following constants during purification: m.p. 68°, $(\alpha)_D$+ 1.45°; m.p. 73°, $(\alpha)_D$— 53.37°; and m.p. 75°, $(\alpha)_D$— 4.49°. On the basis of this initial positive specific rotation, it was suggested (5) that the product obtained by the zinc dust distillation of cholesterol was a mixture of cholesterilene of high negative specific rotation and (dextrorotatory) 2,4 cholestadiene. Cholesterilene was prepared according to the method of Fantl (3) but a specific rotation more negative than — 60° was not observed for the samples obtained which had been purified by repeated recrystallizations.

A mixture of cholesterol and zinc dust was heated under reduced pressure and the reaction product was separated into a dextrorotatory alcohol insoluble fraction which was unaffected by treatment with alcoholic hydrochloric acid and weakly dextrorotatory alcohol soluble needles which yielded strongly laevorotatory needles when treated with alcoholic hydrochloric acid. The conversion of the weakly dextrorotatory alcohol soluble product into a strongly laevorotatory compound (cholesterilene) indicated that this product was a mixture of cholesterilene and 2,4-cholestadiene since 2,4-cholestadiene (dextrorotatory) is converted in cholesterilene (laevorotatory) by treatment with alcoholic hydrochloric acid (5) and since it could not have been just 2,4 cholestadiene ($(\alpha)_D$+ 168.5° (4)) as the product was only weakly dextrorotatory. Repeated attempts to determine the presence of 2,4-cholestadiene by maleic anhydride condensa-

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tion resulted in the formation of an acidic product but this was probably a mixture of acids from maleic anhydride addition compounds, the separation of which was not accomplished. It was thus indicated that in addition to cholesterilene, at least two dextrorotatory compounds are produced by the heat treatment of cholesterol with zinc dust.

Cholesterilene is only slightly adsorbed from petroleum ether solution on activated alumina and hence colored and other adsorbable impurities (including cholesterol) may be easily removed by this means. During the course of the purification of cholesterilene by repeated recrystallization from ether-methanol and from ethyl alcohol on long standing, it was observed that the crystals changed in crystalline structure and became opaque. An alcohol insoluble fraction was isolated by extraction of these opaque crystals with hot alcohol and also by adsorption on activated alumina from petroleum ether solution followed by extraction with ether. This alcohol insoluble solid was also isolated in variable quantities from cholesterilene which had been heated in the absence of a solvent, by adsorption on activated alumina from petroleum ether solution, extraction of the alumina with ether, and repeated reprecipitation of the extract from ether solution by the addition of methonal to yield a white amorphous solid (m.p. 165° (bloc Maquenne), $(\alpha)^{27}_{D}+$ 24.3° (c, 3.23 in $CCl_4$)). This may be a peroxide of cholesterilene since the analysis indicated the empirical formula $C_{27}H_{44}O_2$ and since it liberated iodine from potassium iodide. In general, the sample of cholesterilene of higher negative specific rotation were more resistant to heat since these samples decreased less in laevorotation and less of the dextrorotatory solid was produced. The specific rotation of samples of cholesterilene occasionally becomes less negative even on recrystallization and samples of cholesterilene prepared by the zinc dust method were found more susceptible to marked drops in negative rotation than cholesterilene prepared by the copper sulfate method. Thus a highly laevorotatory sample of cholesterilene prepared by the copper sulfate method has been observed to drop a few degrees whereas one sample of cholesterilene prepared by the zinc dust method dropped as much as 26° in a single recrystallization. This greatly hinders the purification of samples of cholesterilene since it was not found controllable.

## EXPERIMENTAL

### PREPARATION OF CHOLESTERILENE BY THE COPPER SULFATE METHOD

The reaction product obtained by heating an intimate mixture of equal weights of dry cholesterol and powdered anhydrous copper sulfate in an Erlenmeyer flask placed in an oil bath maintained at 175° or 200° was extracted with alcohol, treated with decolorizing carbon, filtered, and allowed to cool. The crude cholesterilene thus obtained was repeatedly recrystallized from various solvents, chiefly ether-methanol and ethyl alcohol, to yield purified cholesterilene. Equal weights of dry cholesterol and powdered anhydrous copper sulfate in an Erlenmeyer flask provided with an air condenser were also refluxed in xylene or toluene (4 cc. per gm. of cholesterol) on a hot plate and the copper sulfate was separated by decantation and washed with ether. The decanted solution and the ether washings were combined and the solvent was removed by distillation under reduced pressure. The residue was dissolved in hot alcohol, treated with decolorizing carbon, filtered, and cooled. The crude cholesterilene in

petroleum ether solution was passed through a column of activated alumina (Alorco, 50 to 200 mesh, freshly heated at 250° for 2 hours) and repeatedly recrystallized from ether-methanol and alcohol to yield purified cholesterilene. The constants observed for the products obtained are presented in table 1.

TABLE 1. *Physical constants of cholesterilene*

| Reaction temperature in degrees C | Time in hrs. | Crude product | | | | Purified product | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | M.P. in °C | Optical rotation | | | M.P. in °C | Optical rotation | | |
| | | | $(\alpha)_D$ | c | °C | | $(\alpha)_D$ | c | °C |
| 200* ..... | 0.33 | 75-77 | — 85.3° | 3.12 | 25 | 79.5-80 | —104.91° | 3.00 | 25 |
| 175 ..... | 0.75 | 76-77.5 | — 92.5° | 3.46 | 25 | 79.5-80 | —106.2° | 3.20 | 26 |
| 140 (in xylene) .... | 7.00 | 77-78.5 | —101.2° | 3.08 | 23 | 79.5-80 | —118.6° | 3.16 | 24 |
| 111 (in toluene) ... | 30.00 | 77-78 | —103° | 3.17 | 24 | 79.5-80 | —117.5° | 3.31 | 21 |

* The constants observed for the purified product obtained under these conditions are included as previously found (2).

A convenient method for the preparation of practically pure cholesterilene in good yield by the dehydration of cholesterol by means of copper sulfate in xylene was thus developed. From 25 gm. of cholesterol, 16 gm. of cholesterilene ($(\alpha)^{25}_D$— 101.2°) were obtained. This was dissolved in 100 cc. of petroleum ether and passed through an 18 x 380 mm. column of activated alumina. The column was eluted with 150 cc. of petroleum ether and the combined filtrates were concentrated. The residue was crystallized once from alcohol to give 14.8 gm. of colorless cholesterilene ($(\alpha)^{21}_D$— 104.4°) which was then recrystallized until the specific rotation was — 118.6°.

### TREATMENT OF CHOLESTEROL WITH ZINC DUST

In a 50 cc. distilling flask a mixture of 13 gm. of dry cholesterol and 100 gm. of zinc dust (Baker, 82.6 per cent) was heated at 260-5° for 45 minutes under the reduced pressure of a water pump. The reaction product was extracted with a total of 200 cc. of petroleum ether (b.p. 68-77°) and the extract was passed through an 18 x 380 mm. column of activated alumina to remove the color and unchanged cholesterol. The column was eluted with 150 cc. of petroleum ether and the filtrates were combined. The solvent was removed by distillation under reduced pressure to yield 7.7 gm. of a colorless oil ($(\alpha)^{22}_D$+ 5.55° (c, 3.24 in $CCl_4$)). This oil was extracted several times with hot alcohol and the alcohol soluble fraction was removed by decantation. The alcohol insoluble fraction was repeatedly reprecipitated from ether solution with methanol to yield 2.2 gm. of a white amorphous solid which melted at 112° (bloc Maquenne) and $(\alpha)^{24}_D$ was + 70.6° (c, 3.06 in $CCl_4$). This product was unaffected by refluxing for 10 hours in 300 cc. of alcohol containing 4 cc. of concentrated hydrochloric acid.

The alcohol soluble extract was refluxed with 4 cc. of concentrated hydrochloric acid for 10 hours, treated with decolorizing carbon, and cooled to crystallize the product (cholesterilene) in needles (3.2 gm.) (m.p. 75-77°, $(\alpha)^{22}_D$—89.36° (c, 3.10 in $CCl_4$)) which on repeated re-

crystallizations melted at 76-77.5° and $(\alpha)^{24}_D$ was —92.6° (c, 2.92 in $CCl_4$). The highest negative specific rotation observed for a sample of cholesterilene (still impure) prepared by the zinc dust method was — 96.2°. From the alcohol soluble fraction were isolated needles ($(\alpha)^{23}_D$+21.6° (c, 2.92 in $CCl_4$)) which when refluxed with 300 cc. of alcohol containing 4 cc. of concentrated hydrochloric acid yielded cholesterilene in needles (m.p. 75-76.5°, $(\alpha)^{25}_D$— 87.0° (c, 3.63 in $CCl_4$)).

The alcohol soluble fraction was treated with maleic anhydride in benzene, toluene, and xylene solution followed by saponification according to the method of Stavely and Bergmann (5) but a definite melting point for the acidic product obtained was not observed and repeated attempts to purify the product indicated that it was a mixture of acids from maleic anhydride addition compounds. The maleic anhydride addition compound, m.p. 265° with decomposition, was obtained by the same procedure from 2,4-cholestadiene which was prepared by a modification of the method of Stavely and Bergmann (5) and of Skau and Bergmann (4) as follows: In a 125 cc. Erlenmeyer flask provided with an air condenser, a mixture of 5 gm. of dry cholesterol, 5 gm. of activated alumina (200 mesh), and 30 cc. of xylene was refluxed for 8 hours in an oil bath. Petroleum ether was added to the reaction product and the solution was passed through an 18 x 210 mm. column of activated alumina (50 to 200 mesh) which was then eluted with petroluem ether. The combined filtrates were concentrated and the residue was crystallized from alcohol to yield 0.9 gm. of 2,4-cholestadiene, m.p. 66-7°, $(\alpha)^{21}_D$+ 142°, which on recrystallization from ether-methanol melted at 67-8° and $(\alpha)^{24}_D$ was + 158.6°.

### EFFECT OF HEAT ON CHOLESTERILENE

A sample of cholesterilene prepared by the copper sulfate method with a specific rotation of —103.8° was found to have a specific rotation of — 101.2° after heat treatment at 100° for 14 days. In a cork stoppered vial 0.545 gm. of cholesterilene prepared by the copper sulfate method with a specific rotation of —84.7° was heated at 100° for 9 days and the specific rotation was found to have become considerably less negative ($(\alpha)^{26}_D$— 2.88° (c, 5.45 in $CCl_4$)). The contents of the vial were dissolved in petroleum ether and passed through a column of activated alumina. The alumina was extracted with ether to give 0.229 gm. of a pale yellow oil ($(\alpha)^{25}_D$+ 24.0° (c, 2.29 in $CCl_4$)) which when repeatedly recrystallized from ether solution with methanol yielded a white amorphous solid (m.p. 165° (bloc Maquenne), $(\alpha)^{27}_D$+ 24.3° (c, 3.23 in $CCl_4$)), the analysis of which indicated the empirical formula $C_{27}H_{44}O_2$ and which liberated iodine from potassium iodide and gave molecular weight values of 355, 361, and 359 (camphor) with the evolution of a gas.

Anal.: Calc'd for $C_{27}H_{44}O_2$: C, 80.92; H, 11.08.
Found: C, 81.15, 80.67, 81.00; H, 11.10, 11.08, 11.21.

Likewise a sample of cholesterilene prepared by the zinc dust method with a specific rotation of — 83.2° when heated at 100° for 24 hours gave a brown glassy solid with a specific rotation of — 7.5° from which the above dextrorotatory solid was obtained. The use of adsorption on activated alumina is also of value in the purification of cholesterilene. Thus the ether extract (yellow oil) of an alumina column through which had

been passed a petroleum ether solution of the reaction product obtained by heating cholesterol with copper sulfate at 200° for 20 minutes had a low specific rotation ($(\alpha)^{28}_D + 0.85°$ (c, 1.61 in $CCl_4$)) whereas the cholesterilene from the filtrate of the column had a specific rotation of $-82.1°$.

## SUMMARY

In the preparation of cholesterilene by the copper sulfate method, the conditions employed were found to be of more importance than the subsequent purification of the product obtained. Although cholesterilene (3,5-cholestadiene) of the highest attained purity is obtained by the pyrolytic decomposition of cholesteryl methyl xanthogenate and by the action of alcoholic hydrochloric acid on allo- or epiallocholesterol, practically pure cholesterilene is most conveniently prepared in good yield by the dehydration of cholesterol with anhydrous copper sulfate.

Heat treatment of cholesterol with zinc dust produces cholesterilene and two dextrorotatory products, one of which was indicated to be 2,4-cholestadiene. 2,4-Cholestadiene was prepared by the dehydration of cholesterol with alumina in xylene. Heat treatment of cholesterilene was found to produce a substance with an empirical formula of $C_{27}H_{44}O_2$ which may be a peroxide of cholesterilene since it liberated iodine from potassium iodide.

## REFERENCES

1. Eck, J. C., and R. L. Van Peursem
1939. The dehydration of cholesterol. Iowa State Coll. J. Sci., 113:115-128.

2. Eck, J. C., R. L. Van Peursem, and E. W. Hollingsworth
1939. The preparation of 3,5- and 4,6-cholestadienes and a study of cholesterilene and of "7-dehydrocholestene isomer." J. Am. Chem. Soc., 61:171-174.

3. Fantl, P.
1926. Zinkstaubdestillation des Cholesterins. Monatsh., 47:251-258.

4. Skau, H. E. and W. Bergmann
1938. The chemistry of unsaturated steroids. IV. The preparation and photochemical oxidation of 2,4-cholestadiene. J. Org. Chem., 3:166-174.

5. Stavely, H. E., and W. Bergmann
1937. The chemistry of unsaturated steroids. II. The preparation and properties of 2,4-cholestadiene. J. Org. Chem., 1:575-579.

# NEW SPECIES AND RECORDS OF SIPHONAPTERA FROM MEXICO

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The following paper is based upon a small collection of fleas taken by Mr. Harry Hoogstraal at Nuevo Leon in Mexico. Of the eight species represented three are semi-cosmopolitan, three are known from Western United States, and two are described as new. The types and other specimens are deposited in the collection of Iowa State College, Ames, Iowa.

## Family HECTOPSYLLIDAE

### *Echidnophaga gallinacea* Westwood

1875 *Sarcopsyllus gallinaceus* Westwood, Ent. Mo. Mag. 11:246.
1906 *Echidnophaga gallinacea* Jordan and Rothschild, Liverpool Univ., Thompson Yates and Johnson Lab. Rep. 7 (n.s.) p. 52.

*Record:* Mexico—Ojo de Agua, Craleano, Nuevo Leon, August 12, 1938, on *"Citellus v. couchi,"* three females; same locality, July 13, 1938, on "pocket gopher," female.

## Family PULICIDAE

### *Pulex irritans* Linnaeus

1758 *Pulex irritans* Linnaeus, Systema Naturae, p. 614.

*Record:* Mexico—Ojo de Agua, Craleano, Nuevo Leon, August 10, 1938, on "dog," female, four males; same locality, August 13, 1938, on *"Citellus v. couchi,"* male.

### *Ctenocephalides canis* Curtis

1826 *Pulex canis* Curtis, British Entomology, Vol. III, No. 114, Figs. A-E, 8.
1930 *Ctenocephalides canis* Stiles and Collins, United States Public Health Ser. Rep. 45:1308.

*Record:* Mexico—Ojo de Aqua, Craleano, Nuevo Leon, August 10, 11, 1938, on "dog," numerous specimens.

## Family DOLICHOPSYLLIDAE

### *Diamanus montanus* Baker

1895 *Pulex montanus* Baker, Can. Ent. 27:132 (Fort Collins, Colorado, on "large mountain gray squirrel *(Sciurus aberti?)*").
1904 *Ceratophyllus acutus* Baker, Invertebrata Pacifica 1:40 (Stanford University, California, on *Spermophilus*).
1904 *Ceratophyllus montanus* Baker, Proc. United States Nat. Mus. 27: 388, Pl. XXII, figs. 7-8, Pl. XXIII, figs. 1-5 (southern Colorado and Arizona on rock squirrel).

1905 *Ceratophyllus montanus* Baker, Proc. United States Nat. Mus. 29:135.
1929. *Ceratophyllus montanus* Jordan, Nov. Zoo. 35:31.
1933 *Diamanus montanus* Jordan, Nov. Zoo. 39:73.

*Record:* Mexico—Ojo de Aqua, Craleano, Nuevo Leon, August 11, 1938, on "dog," female; same locality, August 10-15, 1938, on *"Citellus v. couchi,"* numerous specimens.

Since this species is widely distributed throughout southwestern United States where it parasitizes the ground squirrel in particular, its occurrence in northern Mexico is not surprising.

*Pleochaetis sibynus* Jordan

Plate I, Fig. 3

1925 *Ceratophyllus sibynus* Jordan, Nov. Zoo. 32:110, fig. 42 (Paradise, Arizona, on "skunk").
1933 *Pleochaetis sibynus* Jordan, Nov. Zoo. 39:77.

*Male.* Frontal tubercle distinct and acuminate. Preantennal region of head armed with two rows of bristles; upper row consisting of seven bristles of various sizes, lower row consisting of three very long bristles. Genal process acuminate. Labial palpus reaching almost to the apex of the fore coxa. Second antennal segment armed with a number of very short bristles. A series of small setae along posterior margin of antennal groove. Postantennal region of head armed with three rows of bristles; first row consisting of two or three, second row of four or five, third row of five or six bristles. Pronotum armed with a single row of alternating long and short bristles and a ctenidium of 19 or 20 spines. Meso- and metanotum each armed with a posterior row of long bristles anterior to which are two or three rows of much shorter bristles. Each abdominal tergite armed with two rows of bristles, the anterior tergites further armed with one or two short stout dorsal teeth on a side. Fifth tarsal segment of each leg with five pairs of lateral plantar bristles of which the basal pair is strongly displaced towards the median line. *Modified segments.* Movable finger variable in shape; of the five specimens at hand, three correspond with the figure published by Jordan in his original description while the other two show marked similarity to Jordan's *P. equatoris*[1] in the structure of the movable finger as is demonstrated by Plate I, fig. 3. Posterior margin of movable finger armed with three long bristles and a stout spiniform. Process of clasper more or less triangular in shape, its apex armed with three small bristles. Manubrium short, blunt apically. Penis broad and blade-like, terminating in a curved process; spring long but not completing more than one turn. Sternite VIII not expanded apically; bearing a number of short bristles along the posterior margin and two more robust ones apically.

*Records:* Mexico—Ojo de Aqua, Craleano, Nuevo Leon, July 20, 24, and August 10, 1938, on *"Peromyscus,"* four males; Cerro Potosi, Nuevo Leon, 12,500 ft., July 28, 1938, on *"Microtus,"* male.

[1] Nov. Zoo., 38:344, Fig. 63, 1933 (Ecuador, on *Sigmodon* sp.).

*Foxella mexicana*, n. sp.

Plate I, Figs. 1, 4

*Female.* Frontal tubercle minute. Preantennal region of head armed with two rows of bristles; both upper and lower rows armed with about six bristles. Genal process acuminate. Labial palpus reaching to the apex of the fore coxa. Second antennal segment armed with about ten long bristles which extend beyond the apex of the third antennal segment. A series of small setae along the posterior margin of the antennal groove. Postantennal region of head armed with a single bristle in addition to the marginal row of six or seven (Plate I, fig. 1). Pronotum armed with a single row of alternating long and short bristles and a ctenidium of 24 or 25 spines. Mesonotum armed with two distinct rows of stout bristles anterior to which are numerous much weaker ones. Metanotum armed with only two rows of bristles. Each abdominal tergite armed with a row of exceedingly long bristles anterior to which are two irregular rows of much shorter ones. Fifth tarsal segment of each leg armed with five pairs of lateral plantar bristles. Sternite VII with a sinus ventally. Head of receptaculum seminis round, about as wide as long; tail not longer than the head. For further details concerning the structure of the female genitalia see Plate I, fig. 4.

*Type material.* Female holotype and female paratype collected July 13, 1938, from "pocket gopher" at Cerro Potosi, Nuevo Leon, Mexico.

This new species may be readily separated from *Foxella ignota* (Baker) by the distinctive chaetotaxy of the head and by the genitalia.

*Malareus jordani*, n. sp.

Plate I, Fig. 2

*Male.* Frontal tubercle prominent, acuminate. Preantennal region of head armed with an ocular row consisting of three bristles above which is a series of three shorter bristles near the antennal groove. Genal process acuminate. Labial palpus extending beyond the apex of the fore coxa. Second antennal segment with several short bristles which barely reach to middle of third antennal segment. A series of small setae along posterior margin of antennal groove. Postantennal region of head armed with a marginal row of bristles and a long stout bristle near the second antennal segment above which is a much shorter one. Pronotum armed with a row of alternating long and short bristles and a ctenidium of about 17 spines. Meso- and metanotum each armed with a posterior row of long bristles anterior to which is another row of much shorter bristles. Each abdominal tergite armed with two rows of bristles, the anterior tergites furtherarmed with one or two short stout dorsal teeth on a side. Fifth tarsal segment of each leg armed with five pairs of lateral plantar bristles of which the basal pair is strongly displaced towards the median line. *Modified segments.* Process of clasper broad and lobular armed with four short bristles at the apex. Movable finger broad, concave at both margins, armed with five bristles on the posterior margin of which the basal three are stout spiniforms. Another spiniform, somewhat shorter than the rest, located near the second from the basal spiniform (Plate I, fig. 2). Manubrium blunt distally. Penis broad and heavy, not acuminate distally;

spring very short not completing a single turn. Sternite VIII armed with two bristles located on posterior margin some distance below apex.

*Type material.* Male holotype collected July 20, 1938, from "*Peromyscus*" at Cerro Potosi, Nuevo Leon, Mexico.

This species closely resembles *M. bitterrootensis* (Dunn and Parker) from Montana and *M. euphorbi* (Rothschild) from British Columbia. From these species it may be readily separated by the details of genitalia (Plate I, fig. 2) as well as other structures.

### Family ISCHNOPSYLLIDAE

### *Sternopsylla texana*, C. Fox

1914 *Ischnopsyllus texanus* C. Fox, United States Public Health Ser. Hyg. Lab. Bull. 97:16, Pl. V, figs. 6-8 (Pecos, Texas, on bat, "*Nyctenomus mexicanus*")

1921 *Sternopsylla texana* Jordan and Rothschild, Ectoparasites 1:158.

*Record:* Mexico—Cerro Potosi, Nuevo Leon, August, 1938, on "*Leptonycteris nivalis*," three females.

### PLATE I

### EXPLANATION OF PLATE I

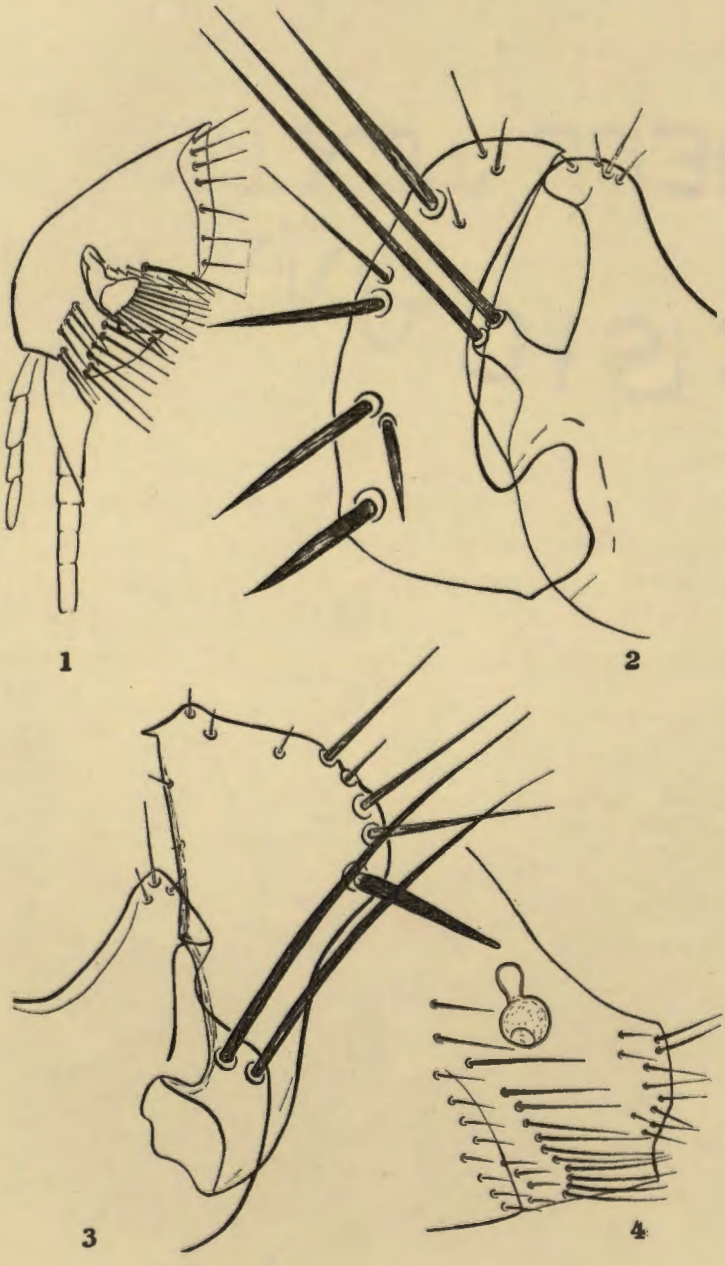
FIG. 1. *Foxella mexicana*, n. sp., female, head.

FIG. 2. *Malareus jordani*, n. sp., male, movable finger and process of clasper.

FIG. 3. *Pleochaetis sibynus* (Jordan), male, movable finger and process of clasper.

FIG. 4. *Foxella mexicana*, n. sp. female, receptaculum seminis and sternite VII.

PLATE I



# NOTES ON TYPHA ANGUSTIFOLIA L. IN IOWA[1]

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Received June 21, 1939

While making a survey in Clay and Palo Alto Counties of the plant cover sheltering waterfowl (Plate I), it was observed that the cat-tail population was not uniform. In shallow lakes and marshes in the vicinity of Ruthven during the past five years (1933 to 1938), plants of the type described as *Typha angustifolia* L. have been more widespread than *T. latifolia* L. Dr. W. A. Anderson has located a colony of *Typha angustifolia* from which specimens have been collected in the vicinity of Lake Okoboji in Dickinson County, and Dr. H. S. Conard reported to the writer a collection from Rush Lake in Osceola County. The name only appears for the first time in Conard's Key to Plants of Iowa (Grinnell Flora 5th ed. 1939), though no written discussion of *T. angustifolia* in Iowa has been noted. The occurrence of *T. latifolia* has been reported in several adjoining states; in Missouri (7), local in Saline County, in the shallow water of a spring-fed lake; in Wisconsin (2), occurring in marshes in the vicinity of Madison; in central Kansas (3), in salt marshes; in Illinois (8), abundant locally in marshes surrounding Stony Island, rare at other points in the vicinity of Chicago; locally in South Dakota (6), in water which is more or less alkaline.

*T. angustifolia* (Plate IV) is less prominent in Clay and Palo Alto Counties than its variety *T. angustifolia* L. var. *elongata* (Dudley) Wiegand (Plate II, fig. 2) which occurs in dense colonies and borders on the north side of Mud Lake, along the south and west shores of Lost Island Lake and its outlet (Plate III), around the west bay and shore of Trumbull Lake, in colonies in Elk and Virgin Lakes and in large areas particularly along the east third of Round Lake. In the summer of 1938 a large colony of *T. angustifolia* var. *elongata* was located in New Lake, an oxbow lake in Woodbury County, about one-half mile west of the town of Salix.

Table 1 compares the chief characters that distinguish the main types of Typha thus far observed. However, some intermediate types which appear to be hybrids cannot yet be included because of insufficient data.

In the shallow lakes of Clay and Palo Alto Counties the cat-tail phase of the emergent flora advances or recedes with the rise or fall of the water level which varies in depth from a few inches to two or three feet around the cat-tails. In 1935 and 1936 some stands were left by the receding water on mud flats for a time. The position of the colonies is affected temporarily through cutting by muskrats which assist in the cycle of succession.

In 1937 many of the cat-tails were attacked by micro-organisms which caused rotting of the rootstock after which the plants collapsed and sank under the water. The stands of the narrow-leaved cat-tails during the five years observed in such shallow lakes as Round, Mud, and Trumbull maintained themselves largely in vigorous condition and covered a

[1]Journal Paper No. J647 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 366.

TABLE 1. *Comparative descriptive characteristics of Typha*

| | Vegetative structures | | | Inflorescence | | | |
|---|---|---|---|---|---|---|---|
| Species | Height | Stem | Leaves | Size of spike in fruit | Color | Pollen | Bractlets |
| Typha angustifolia | 1–1.5 m. | Slender | Convex on back, lower leaves 3–7 mm. wide | 8–13 cm. l. 10–17 mm. in diam. | Cinnamon with black markings | Single | Pistillate fl. with bractlets |
| Typha angustifolia var. elongata | 2–3.5 m. | Slender | Flat, lower leaves 9–15 mm. wide | 15–25 cm. l. 20–23 mm. in crown | Cinnamon with black markings | Single | Pistillate fl. with bractlets |
| Typha latifolia | 1–2 m. | Stout | Flat, lower leaves 9–15 mm. wide | 8–15 cm. l. at least 25 mm. in diam. | Dark brown with black markings | In 4's | Pistillate fl. without bractlets |
| Typha (intermediate types) | In general aspect like T. angustifolia or its variety | | | | | In 4's | |

greater area than *T. latifolia* (Plate II, fig. 1) which usually occurs in shallower water, or in the fresh water of hilltop springs known as hanging bogs. Associated with *T. angustifolia* and its variety were some narrow-leaved forms in which the gross aspects of the spike resembled the narrow-leaved type. The pollen grains, however, occur in fours, which structure is characteristic of *T. latifolia*. Other forms in stature intermediate between the two species were seen. These structural variations seem indicative of hybridity.

In the herbarium of Iowa State College is a folder of plants designated Typha. These specimens, in their macroscopic morphology are typical of *T. angustifolia* or its variety, but microscopic inspection shows that they have tetrad pollen grain. This rather robust type which, judging from herbarium specimens, is more or less widespread inland, appears to be a product of the inland environment where it exists with its probable parent species. The relative distribution of these types inland will be revealed, perhaps, by more extended field work and laboratory study.

The water of the lakes mentioned has a pH of 7.5 to 8.5 in the neighborhood of the cat-tails, though higher readings have been made elsewhere. The bottom of the lake is heavily silted (4) through long action of erosional forces operating on the adjacent land. The soil is highly calcareous through the accumulation of plant residues for long periods, and by leaching of salts from the upland (1).

That *T. angustifolia* and its variety have not been more frequently collected is perhaps accounted for by their occurrence in the deeper water of marshes and lakes than *T. latifolia* and by the prevailing idea that *T. angustifolia* is a plant restricted to coastal salt marshes. According to records, the narrow-leaved cat-tail is recurrent inland in saline areas or in the water of alkaline lakes, the soils of which are highly calcareous and where there is poor drainage.

Svenson (11), in a survey of effects of post-pleistocene submergence, finds that the botanical evidence of oceanic submergence offered by early writers as an explanation for the presence of halophytic plants is of little value in accounting for their distribution inland. He states that inland areas with impeded drainage where the underlying rocks are calcareous afford a favorable habitat. In Clay (9) and Palo Alto (10) Counties the underlying rock lies so far below the surface that it does not affect the character of the soils and the waters of the shallow lakes and ponds are derived from surface runoff rather than from subterranean sources. However, the poor drainage resulting in the retention of accumulated vegetative residues which decompose into peat and muck, as well as the products of silting and leaching from higher ground contributes, to a highly calcareous substratum. In these areas such dwellers in brackish costal marshes as the narrow-leaved cat-tail are able to persist inland. Metcalf (5) in researches on North Dakota lakes has determined the total concentration of salts in 75 lakes from which the vegetation is listed. From these data it appears that some of the waters of higher concentrations support only plants that range in America from fresh through brackish, to salt water, such as *Potamogeton pectinatus* and *Ruppia maritima*. It seems probable, therefore, that high concentrations of soil solutions may have a bearing on the tolerance of inland plants, which are also identified with coastal salt marshes.

The foregoing discussion reports for the first time *Typha angustifolia*

and its variety *elongata* as relatively abundant locally in northern Iowa. Associated with the narrow-leaved types are forms intermediate between *T. angustifolia* or its variety and *T. latifolia.* The inland environment is compared with the coastal marshes. Iowa collections upon which this discussion is based and some others are filed in the herbarium of Iowa State College.

## LITERATURE CITED

1. Brown, P. E.
1936. Soils of Iowa. Iowa Agr. Exp. Sta. Spec. Rept. No. 3:146.

2. Fassett, Norman C.
1930. Preliminary reports on the flora of Wisconsin. Typhaceae. Trans. Wis. Acad. Sci. Arts and Let. 25:183-184.

3. Gates, Frank C.
1933. Wild flowers in Kansas. Report of the Kansas State Board of Agr. Topeka, Kansas.

4. Report of State Highway Commission
1916. Iowa Lakes and Lakebeds.

5. Metcalf, Franklin P.
1931. Wild duck foods of North Dakota lakes. U.S.D.A. Tech. Bul. 221.

6. Over, Willliam H.
1932. Flora of South Dakota. Univ. of S.D. Vermilion.

7. Palmer E. J., and J. A. Steyermark
1935. An annotated catalogue of the flowering plants of Missouri. Ann. Mo. Bot Gard. 22:375-758.

8. Pepoon, H. S.
1927. An annotated flora of the Chicago area. Chicago Academy of Sciences. Nat. Hist. Surv. Bul. 8.

9. Stevenson, W. H., P. E. Brown, with the assistance of G. E. Corson, T. H. Benton and M. E. Olson
1919. Clay County soils. Soil Surv. of Iowa. Reports No. 12.

10. Stevenson, W. H., P. E. Brown, with the assistance of A. M. O'Neal, Jr., L. W. Forman and W. G. Baker
1922. Palo Alto County soils. Soil surv. of Iowa. Report No. 22.

11. Svenson, H. K.
1927. Effects of post-pleistocene marine submergence in eastern North America. Rhodora, 29:41-48, 57-72, 87-93, 105-114.

## PLATE I.

Waterfowl feeding on duckweed in a narrow-leaved cat-tail marsh. Outlet of Lost Island Lake, Clay County.

PLATE I.

PLATE II.

FIG. 1. A zone of *Typha latifolia* L. in shallow water in Dewey's Pasture, Clay County.

FIG. 2. A colony of *Typha angustifolia* L. var. *elongata* (Dudley) Wiegand in Mud Lake, Dewey's Pasture, Clay County.

PLATE II.

PLATE III.

*Typha angustifolia* L. var. *elongata* (Dudley) Wiegand (cat-tails) in Barringer's Slough, Clay County.

PLATE III.

PLATE IV.

The narrow-leaved cat-tail (*Typha angustifolia* L.)—a service and shelter plant for waterfowl, growing in Round Lake, Clay County.

PLATE IV.

# THE EFFECT OF TREATED FATS ON VITAMIN A POTENCY[1]

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Fridericia (1) has reported that hydrogenated whale oil, when mixed with fat containing vitamin A, causes destruction of this vitamin. Ordinary whale oil, according to Drummond (2) and Fridericia (1), contains this dietary factor; whereas, hydrogenated whale oil does not contain this vitamin. It is therefore evident that hydrogenation causes some chemical change in the oil resulting in the formation of certain products that destroy vitamin A. Fridericia also states that heated lard causes destruction of vitamin A, when mixed with fat containing this vitamin; whereas, unheated lard does not have this effect. He reports, furthermore, that certain hydrogenated and nonhydrogenated vegetable fats, when mixed with fat containing vitamin A, do not cause destruction of this vitamin. Powick (3) found that vitamin A in butterfat was destroyed when fed with rancid fats, and Mattill (4) also showed that both vitamins A and E were destroyed by rancid fats. Whipple (5) found that vitamin A in cod liver oil is destroyed as rancidity develops in the oil. For several years Nelson, Nelson, and Lowe (6) have published results on the effect of heated fats on vitamin A. Recently Lease, Lease, Weber, and Steenbock (7) have published work on the effect of rancid and heated fats on carotene and vitamin A. They cite evidence to show that fats which had been heated at such temperatures as are frequently used in cooking destroyed vitamin A to some extent.

Since heated lard causes destruction of vitamin A when mixed with fat containing this vitamin, it seemed important to ascertain whether or not vegetable fats act similarly. Fridericia (1) did not study the action of either heated hydrogenated vegetable fats or heated nonhydrogenated vegetable fats upon vitamin A. Experiments were therefore instituted in order to answer the following questions: First, what effect do heated and unheated fats have on vitamin A activity of butterfat when mixed with the latter fat? Secondly, from a practical standpoint, will baking with lard or other fats cause destruction of vitamin A in the baked product? No distinction in this work has been made between vitamin A and carotene; consequently the observed effect is that of treated fats on vitamin A potency or activity.

## EXPERIMENTAL

All of the experiments were performed on rats weighing from 45 to 55 gms. when placed on the various rations; they were obtained from the stock colony maintained by the Chemistry Department, Iowa State College. They were housed in wooden frame cages, the sides of which were composed of hardware cloth; galvanized pans served as bottoms and wood

[1] Journal Paper No. J-661 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 328.

shavings were employed as litter. Six rats, three males and three females, were placed in each lot; they were weighed weekly and cared for daily. The following basal ration was employed in the tests: casein 200 gms., salts 50 gms., yeast 100 gms., and dextrin 450 gms. The casein was prepared by washing the commercial product daily with 0.15 per cent acetic acid until free of vitamin A. The salt mixture was essentially the one employed by McCollum and Davis (8) and designated by them, salt mixture 185. It contained additional small quantities of copper and manganese salts together with a small amount of potassium iodide. The yeast was a dried product obtained from Standard Brands, Inc. Dextrin was made by treating starch with 0.37 per cent citric acid solution and autoclaving at 15 pounds pressure for three hours.

The data in this paper will be considered under two heads: First, the effect of individual fats, heated and unheated, on the vitamin A activity of butterfat when mixed with the latter fat; and, secondly, the effect of bakng on the vitamin A activity of butter or egg yolk in cookies containing different fats.

### THE EFFECT OF HEATED AND UNHEATED FATS ON VITAMIN A POTENCY

The following four rations, similar to those employed by Fridericia (1), were used in this study: Ration I consisted of 800 gms. of the basal ration and 200 gms. of butterfat; ration II consisted of 900 gms. of the basal ration plus 100 gms. of butterfat; ration III was composed of 800 gms. of the basal ration plus 100 gms. of butterfat and 100 gms. of the fat investigated; and ration IV consisted of 800 gms. of the basal ration, 100 gms. of butterfat, and 100 gms. of the corresponding heated fat undergoing investigation. The latter had been heated 24 hours in a thin layer (one-eighth inch thick) at 102° C. to 105° C. The butterfat and the fat tested were mixed at temperatures slightly above the melting points of the two fats. The butterfat employed in the studies was prepared by heating butter on a steam plate to the melting point, following which the fat was promptly decanted from the salts, water, and curd. Rations I and II served as controls for all of the fats and oils; they were not repeated for each test, although they were employed a total of three times. Growth of the animals was normal on rations I and II. Rations III and IV were employed for each fat studied.

The results of this series of experiments are given in table 1. The effect of heated fats upon vitamin A activity was made evident by the poor growth of the animals receiving the heated fats and the development of xerophthalmia in the animals; each lot was continued on experiment for at least 12 weeks, or until death intervened. The plus sign under growth signifies that body weight increased steadily, and the negative sign signifies that loss of weight occurred after different lengths of time, and that the animals died. The figures under xerophthalmia designate the numbers of animals showing symptoms of the disease and, since six animals were employed in each lot, the remaining animals in the lots died without displaying any evidence of the malady. The letters N. D. signify that vitamin A activity was not destroyed, and the letter D signifies that destruction of this vitamin occurred. U. V. indicates that the animals were exposed to ultra-violet light for five minutes daily. The term storage refers to animal fats kept under specified conditions for varying lengths of time. The storage animal fats (experiments 14, 15, and 16) were kept in a cool place.

TABLE 1. *The effect of heated and unheated fats on vitamin A activity of butterfat*

| Exp. No. | Name of fat in text | Basic fat material | Trade name, description or source | Growth U.H. | Growth H. | Xerophthalmia U.H. | Xerophthalmia H. | Effect U.H. | Effect H. |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Veg. fat 1 | cottonseed oil | Snowdrift | + | − | 0 | 3 | N.D. | D. |
| 2. | Veg. fat 2 | cottonseed oil | Crisco | + | − | 0 | 3 | N.D. | D. |
| 3. | Veg. fat 3 | olive oil | Pompeian | + | − | 0 | 2 | N.D. | D. |
| 4. | An. fat 4 | lard | commercial | + | − | 0 | 4 | N.D. | D. |
| 5. | An. fat 4 U.V. | lard | commercial | + | − | 0 | 5 | N.D. | D. |
| 6. | An. fat 5 | lard | Wilson B. P. S. | + | − | 0 | 5 | N.D. | D. |
| 7. | An. fat 6 | lard | Wilson O. K. | + | − | 0 | 4 | N.D. | D. |
| 8. | An. fat 7 U.V. | lard | Wilson B. P. S. | + | − | 0 | 5 | N.D. | D. |
| 9. | An. fat 8 U.V. | lard | Wilson O. K. | + | − | 0 | 5 | N.D. | D. |
| 10. | An. fat 9 U.V. | lard | Wilson B. P. S. | + | − | 0 | 5 | N.D. | D. |
| 11. | An. fat 10 U.V. | lard | Wilson O. K. | + | − | 0 | 5 | N.D. | D. |
| 12. | An. fat 11 U.V. | hydrogenated lard | Clix | + | − | 0 | 5 | N.D. | D. |
| 13. | An. fat 12 | lard | R. P. (50 per cent leaf; 50 per cent back) | + | − | 0 | 6 | N.D. | D. |
| 14. | An. fat 13 | storage lard | Wilson O. K. | + | − | 0 | 2 | N.D. | D. |
| 15. | An. fat 14 | storage lard | Wilson B. P. S. | + | − | 0 | 5 | N.D. | D. |
| 16. | An. fat 15 | rancid storage lard | commercial | − | − | 4 | 3 | D. | D. |
| 17. | An. fat 16 | drip lard | French Oil Machinery Co., Piqua, Ohio | + | − | 0 | 4 | N.D. | D. |
| 18. | An. fat 12 | lard (105°) | R. P. | + | − | 0 | 2 | N.D. | D. |
| 19. | An. fat 12 | lard (80°) | R. P. | + | + | 0 | 0 | N.D. | N.D. |
| 20. | An. fat 12 | lard (67°) | R. P. | + | + | 0 | 0 | N.D. | N.D. |
| 21. | An. fat 12 | lard (37.5°) | R. P. | + | + | 0 | 0 | N.D. | N.D. |
| 22. | Oleo 21 | oleomargarine | Hollynut | + | + | 0 | 0 | N.D. | N.D. |
| 23. | Oleo 21 S | oleomargarine | Hollynut | + | + | 0 | 0 | N.D. | N.D. |
| 24. | Oleo 22 | oleomargarine | Bestonut | + | + | 0 | 0 | N.D. | N.D. |
| 25. | Oleo 22 S | oleomargarine | Bestonut | + | + | 0 | 0 | N.D. | N.D. |
| 26. | Oleo 21 | oleomargarine | Hollynut | + | + | 0 | 0 | N.D. | N.D. |
| 27. | Oleo 21 S | oleomargarine | Hollynut | + | + | 0 | 0 | N.D. | N.D. |
| 28. | Oleo 23 S | oleomargarine (mainly coconut oil) | Gem Nut | + | + | 0 | 0 | N.D. | N.D. |
| 29. | Oleo 24 S | oleomargarine (mainly oleo oil and neutral lard) | Premium | + | − | 0 | 2 | N.D. | D. |
| 30. | Oleo 25 S | oleomargarine (cottonseed oil) | Virginia Belle | + | − | 0 | 3 | N.D. | D. |

TABLE 1. (continued)

| Exp. No. | Name of fat in text | Basic fat material | Trade name, description or source | Growth U.H. | Growth H. | Xerophthalmia U.H. | Xerophthalmia H. | Effect U.H. | Effect H. |
|---|---|---|---|---|---|---|---|---|---|
| 31. | Oleo 26 | oleomargarine (coconut oil and cottonseed oil) | Nutola | + | — | 0 | 1 | N.D. | D. |
| 32. | Oleo 27 | oleomargarine (oleo oil, neutral, cottonseed oil) | Silver Churn | + | — | 0 | 4 | N.D. | D. |
| 33. | Oleo 28 | oleomargarine (partially hydrogenated cottonseed oil) | Good Luck | + | — | 0 | 4 | N.D. | D. |
| 34. | An. fat 29 | green lard | Wilson | + | — | 0 | 5 | N.D. | D. |
| 35. | An. fat 30 | green lard | Swift | + | — | 0 | 3 | N.D. | D. |
| 36. | An. fat 31 | butterfat | College | + | — | 0 | 4 | N.D. | D. |
| 37. | Veg. fat 32 | coconut oil | commercial | + | + | 0 | 0 | N.D. | N.D. |
| 38. | Veg. fat 33 | coconut oil | commercial | + | + | 0 | 0 | N.D. | N.D. |
| 39. | Veg. fat 34 | coconut oil | commercial | + | + | 0 | 0 | N.D. | N.D. |
| 40. | Veg. fat 35 | coconut oil | commercial | + | — | 0 | 6 | N.D. | D. |
| 41. | Veg. fat 36 | coconut oil | commercial | + | — | 0 | 5 | N.D. | D. |
| 42. | Veg. fat 37 | coconut oil | commercial | + | — | 0 | 3 | N.D. | D. |
| 43. | Veg. fat 38 | coconut oil | commercial | + | — | 0 | 4 | N.D. | D. |

Note: R. P. refers to record of performance lard. This lard was obtained from animals used in Project 39: "Swine Performance Record," Iowa Agr. Exp. Sta. Record of performance lard is lard made from equal parts by weight of leaf and back fat. Skins are removed and the fat is ground and rendered in a steam jacketed open kettle at a temperature not to exceed 237° F.

Commercial refers to fats in bulk purchased on the local market.

Coconut oil was purchased locally and the coconut oils in the different experiments were different oils from different sources.

An. fat refers to animal fat, and Veg. fat designates vegetable fat.

U.H. signifies unheated, and H. heated.

O. K. refers to open kettle lard, and B. P. S. to bleached prime steam lard.

S signifies that the fat was decanted from salts, water, and curd.

U. V. signifies that the animals were exposed to ultraviolet light for five minutes daily.

N.D. signifies that vitamin A activity was not destroyed; D signifies that vitamin A activity was destroyed.

Green lard is lard possessing a distinct green color.

Unless otherwise mentioned, all of the heated fats were subjected to a temperature of 102° C. to 105° C.

Open kettle storage lard (experiment 14) and bleached prime steam storage lard (experiment 15) were kept in a cooler at 0° C. to 5° C. for seven months and subsequently in a cool room at a temperature between —6° C. and 15° C. for three months. The rancid commercial storage lard (experiment 16) was kept for 10 months in a cool room at a temperature between —6° C. and 15° C.

### EXPERIMENTS ON BAKED PRODUCTS

The experiments were performed to ascertain the extent of vitamin A destruction during baking. Cookies served as a convenient baked product to study. Egg yolk or butterfat served as the source of vitamin A in the cookies. The baking temperature was 170° C. for 11 minutes. The size of the cookies was maintained as nearly constant as possible, and the entire baking process was regulated in such a way that errors were reduced to a minimum. Five batches of egg cookies were prepared; the formulae of the cookies are given in table 2. Egg yolk served as a source of vitamin A in these cookies. Batches I and II were prepared for control purposes.

TABLE 2. *Formulae of cookies containing egg yolk as the sole source of vitamin A*

| Batch I | | Batch II | | Batch III | |
|---|---|---|---|---|---|
| White flour | 276 | White flour | 276 | White flour | 276 |
| Sugar | 125 | Sugar | 125 | Sugar | 125 |
| Water to roll. | | Egg yolk | 72 | Egg yolk | 72 |
| | | Water to roll. | | Animal fat 5* | 110 |
| | | | | Water to roll. | |

| Batch IV | | Batch V | |
|---|---|---|---|
| White flour | 276 | White flour | 276 |
| Sugar | 125 | Sugar | 125 |
| Egg yolk | 72 | Egg yolk | 72 |
| Vegetable fat 2* | 110 | Animal fat 11* | 110 |
| Water to roll. | | Water to roll. | |

* See table 1 for nature of fats used. All figures in tables 2, 3, 4, and 5 are expressed in grams.

Five lots of rats were placed on rations containing the five different cookies the ingredients of which are given in table 2. The rations had the composition shown in table 3.

TABLE 3. *Composition of rations containing the egg cookies*

| Lot I | | Lot II | | Lot III | |
|---|---|---|---|---|---|
| Casein | 200 | Casein | 200 | Casein | 200 |
| Salts | 50 | Salts | 50 | Salts | 50 |
| Yeast | 100 | Yeast | 100 | Yeast | 100 |
| Olive oil | 147 | Olive oil | 147 | Cookies Batch III | 650 |
| Cookies Batch I | 553 | Cookies Batch II | 640 | | |

| Lot IV | | Lot V | |
|---|---|---|---|
| Casein | 200 | Casein | 200 |
| Salts | 50 | Salts | 50 |
| Yeast | 100 | Yeast | 100 |
| Cookies Batch IV | 650 | Cookies Batch V | 650 |

The rats in lot I lost weight, exhibited xerophthalmia, and died in from four to six weeks' time. Rats in lot II grew normally. It is evident that the egg yolk supplied all of the vitamin A in the cookies. Animal fat 5, vegetable fat 2, and animal fat 11 when incorporated in the cookies containing the egg yolk as the sole source of vitamin A did not cause destruction of the vitamin A in the baked product, nor was the vitamin destroyed in the cookies containing egg yolk but no added fat. The experiments were continued for as long as 18 weeks; data on growth and xerophthalmia are given in table 6 (experiment A, lots I to V, inclusive). Five batches of cookies with butter as the sole source of vitamin A were then baked; they had the formulae shown in table 4. The butter employed in the experiments recorded in this paper was purchased from the Dairy Industry Department, Iowa State College.

TABLE 4. *Formulae of cookies containing butter as the sole source of vitamin A*

| Batch VI | Batch VII | Batch VIII |
|---|---|---|
| White flour .....276 | White flour .....276 | White flour .....276 |
| Sugar .....125 | Sugar .....125 | Sugar .....125 |
| Butter .....110 | Butter ..... 55 | Butter ..... 55 |
| Water to roll. | Animal fat 5* ..... 55 | Vegetable fat 2* .... 55 |
| | Water to roll. | Water to roll. |

| Batch IX | Batch X |
|---|---|
| White flour .....276 | White flour .....276 |
| Sugar .....125 | Sugar .....125 |
| Butter ..... 55 | Animal fat 5* .....110 |
| Animal fat 11* ..... 55 | Water to roll. |
| Water to roll. | |

* See table 1 for nature of fats used in these batches of cookies.

Five lots of rats were placed on rations containing the cookies of table 4. The composition of the rations fed to the rats is given in table 5.

TABLE 5. *Composition of rations containing butter cookies*

| Lot VI | Lot VII | Lot VIII |
|---|---|---|
| Casein .....200 | Casein .....200 | Casein .....200 |
| Salts ..... 50 | Salts ..... 50 | Salts ..... 50 |
| Yeast .....100 | Yeast .....100 | Yeast .....100 |
| Cookies Batch VI ...650 | Cookies Batch VII ..650 | Cookies Batch VIII ..650 |

| Lot IX | Lot X |
|---|---|
| Casein .....200 | Casein .....200 |
| Salts ..... 50 | Salts ..... 50 |
| Yeast .....100 | Yeast .....100 |
| Cookies Batch IX ...650 | Cookies Batch X ....650 |

The animals in lots VI, VII, IX, and X grew for a short time (one-half to two months), then their weights declined, and finally death occurred. Animals in lot VIII grew normally for a period of 16 weeks, when the experiment was terminated. The results of this experiment are given in table 6 (experiment A, lots VI to X, inclusive). A second series of butter

cookies was tested. These cookies were prepared according to the same method and had the same formulae as the first series of butter cookies (table 4). The diets containing the second series of butter cookies had the composition as given in table 5. The experiments on the second series of butter cookies were begun about three and one-half months after the beginning of the first series of butter cookie experiments; the data are recorded in table 6 (experiment B, lots VI to X, inclusive). The results are not in agreement with those obtained on the first series of butter cookies, experiment A, lots VI to X, inclusive. In the first series of butter cookies only lot VIII, receiving vegetable fat 2, grew normally; whereas, in the second series of butter cookies the animals in lots VIII and IX, receiving vegetable fat 2 and animal fat 11, respectively, exhibited normal curves of growth. The other lots ceased to grow after one-half to three months on the rations. The control group, experiment B, lot I, received the same ration as did the animals in lot I, table 3. The experiments on the egg cookies also were repeated, and the results were the same as on the first series of egg cookies. The data on the second series of egg cookies are given in table 6 (experiment B, lots I to V, inclusive).

The butter cookies remaining after experiment A, table 6, was completed were stored at a temperature of 26° C. for 10 months, incorporated into rations having the composition given in table 5, and fed to the animals.

TABLE 6. *Results obtained on the baked products*

| Experiment | Lot I | Lot II | Lot III | Lot IV | Lot V | Lot VI | Lot VII | Lot VIII | Lot IX | Lot X |
|---|---|---|---|---|---|---|---|---|---|---|
| A ..... | −(2) | + | + | + | + | −(3) | −(6) | + | −(4) | −(4) |
| B ..... | −(2) | + | + | + | + | −(3) | −(4) | + | + | −(4) |
| C ..... | −(3) | | | | | −(4) | −(3) | + | −(5) | −(4) |
| D ..... | −(2) | | | | | + | + | −(6) | + | −(2) |
| E ..... | −(2) | | | | | + | + | + | + | −(2) |

+ and − signs designate rate of growth as compared to normal.

Figures refer to the number of animals exhibiting xerophthalmia. The animals were kept on experiment for at least 12 weeks (in some cases as long as 18 weeks) or until death intervened.

The results are recorded in experiment C, table 6. The rats in lot VIII grew normally for 12 weeks, when the experiment was discontinued; the remaining animals ceased to grow at varying times, ranging from one to three months. A third and fourth series of butter cookies were prepared having the same formulae as series I, table 4, and the rations containing the cookies had the composition given in table 5. The third series of butter cookies was kept at 26° C., and enough of each batch of the cookies was made into rations at one time for a three weeks' supply. The data obtained with these cookies are given in table 6 (experiment D, lots VI to X, inclusive). The fourth series of butter cookies was kept in a cold room (—6° C. to 10° C.). Enough of each ration for a three weeks' supply was made each time, but only a one week's supply of the ration was kept in the laboratory, the remainder being kept in the cold room. The results of this experiment are shown in table 6 (experiment E, lots VI to X, inclusive).

The same fats were employed for the preparation of the third and fourth series of butter cookies.

TABLE 7. *Peroxide content of fats*

| Fats | Peroxide value (millimols per 1,000 gms.) |
|---|---|
| Unheated oleo 21 | 3.90 |
| Heated oleo 21 | 62.8 |
| Unheated oleo 23 | 1.04 |
| Heated oleo 23 | 67.3 |
| Unheated oleo 24 | 1.06 |
| Heated oleo 24 | 64.3 |
| Unheated oleo 25 | 2.78 |
| Heated oleo 25 | 66.3 |

## DISCUSSION OF RESULTS

The data in table 1 reveal that most of the heated fats, when mixed with unheated butterfat, cause destruction of vitamin A contained in the latter fat. However, two brands of oleomargarine, designated oleo 21 and oleo 22, which were purchased on the local market, acted differently from the other fats. Our first experiments on both margarines were made on the products as purchased. These margarines when heated and mixed with unheated butterfat did not destroy vitamin A of the butterfat (table 1, experiments 22, 24). It might be well to emphasize at this point that all samples of butterfat employed in the experiments, whose results are given in table 1, were prepared from butter by decanting the fat from water, curd, and salts. Since the two brands of oleomargarine acted differently from the other fats, it was believed that this might be caused by the salts or curd; and that, if these substances were removed, the margarines upon heating might act like the other fats. Consequently, another experiment was performed, in which both margarines were melted and the fat layer decanted; the decanted fats were then heated for 24 hours at 102° C. to 105° C. and mixed with unheated butterfat. It will be observed from table 1, experiments 23 and 25, that vitamin A activity of the butterfat was not destroyed when the margarines so treated were mixed with unheated butterfat. The unheated margarines did not cause destruction of vitamin A activity when mixed with unheated butterfat, as was to be expected. In all cases the fat tested and the butterfat were mixed at a temperature slightly above the melting point of the fats and the mixture added in the proper amount to the ration. In experiments 22, 24, and 26 the margarines were employed as purchased; whereas, in experiments 23, 25, and 27, S signifies that decanted fats were used. It would appear that the two margarines, oleo 21 and oleo 22, act differently from other fats on heating. However, because the results on these margarines were unique, it was deemed advisable to repeat the experiments on decanted and nondecanted oleo 21. It will be observed, table 1, experiments 26 and 27, that the results of the second test on oleo 21, heated and unheated, were the same as those obtained on the first sample. Oleo 22 was not tested again, because it was not available on the local market.

The two oleomargarines acted differently from most of the other fats, for example, animal fat 31, as shown by experiment number 36, table 1. Animal fat 31, after melting and decanting, was heated for 24 hours at

102° C. to 105° C. in a thin layer (one-eighth inch thick) and mixed with unheated butterfat; and it will be observed from the data that vitamin A in the unheated butterfat was destroyed. In view of this difference between certain margarines and certain animal fats, it was decided to test some more margarines. Dr. W. Lee Lewis of the Institute of American Meat Packers supplied us with three different oleomargarines. These margarines are called oleo 23, oleo 24, and oleo 25. Oleo 23 was made principally from coconut oil, oleo 24 was manufactured from fat of which approximately 75 per cent was animal fat (made up of oleo oil and neutral lard) and 25 per cent was cottonseed oil, and oleo 25 was prepared entirely from cottonseed oil. The results are given in table 1, experiments numbers 28, 29, and 30. Xerophthalmia or death resulted in the rats on heated oleo 24 and oleo 25, so that none of the animals fed these margarines survived at the end of nine weeks. All but one of the rats were dead on heated oleo 25 at the end of six weeks, and the remaining animal died at the end of nine weeks. Three of the rats on heated oleo 24 were killed because of middle ear infection, two animals died of xerophthalmia, and the remaining one died without evidence of this disease. One of the animals on heated oleo 23 was killed because of otitis media, but the remainder, at the end of 12 weeks, were in good physical condition. This experiment seems to indicate that margarines composed of coconut oil act differently on heating than margarines composed of certain other fats. All three margarines were decanted after melting, in order to eliminate salts, curd, and water; the tests were performed on the decanted fats.

Naturally, the next problem was to explain the action of the margarine prepared from coconut oil. The margarine made of coconut oil (experiment 28) and three samples of coconut oil (experiments 37, 38, and 39) acted similarly in that none of them cause destruction of vitamin A in butterfat when heated and mixed with unheated butterfat. However, certain other samples of heated coconut oil caused destruction of vitamin A when mixed with butterfat (experiments 40, 41, 42, 43). It was believed that the differences obtained with the various margarines might be caused by the quantity of peroxide, but the analyses in table 7 do not support this contention. It will be observed from the data in this table that heated oleo 23 contained more peroxide than heated oleo 24 and heated oleo 25, but the vitamin A activity of butterfat was not destroyed by heated oleo 23, but the vitamin activity was destroyed by the other two heated margarines. Furthermore, heated oleo 21 contained almost as much peroxide as heated oleo 24, nevertheless the effects of these two margarines on vitamin A activity were markedly different. More convincing evidence that peroxides are not the only factors responsible for the destruction of vitamin A is shown by the effects of animal fats 29 and 30 on this vitamin. Both of these heated fats caused destruction of vitamin A, even though they contained only 52.53 and 56.21 millimols of peroxide per 1,000 grams of fat, respectively. The data, therefore, strongly suggest that some unknown substances, aside from peroxides, are responsible in whole or in part for the effects produced by heated fats, and that the heated coconut oil margarine either contained less of these substances or else it contained substances of unknown function, possibly of the nature of anticatalyzers,which retarded the reaction. The work of Olcovich and Mattill (9) lends support to some of these conclusions. They say that the commonly measured stability of vitamin A (carotene) in different vegetable foods exposed to heat and light

does not depend upon the carotene itself or on the presence of oxygen, primarily, but is dependent upon the presence of other substances. Furthermore, they state: "Since carotene in the solid state is decolorized and rendered inert, physiologically, by heat in the absence of oxygen, and since in solution it undergoes this change even more rapidly under the influence of heat or ultraviolet radiation, it follows that the resultant achroocarotene is not a product of oxidation but rather of an intramolecular rearrangement, or possibly of polymerization. Inasmuch as hydroquinone delays the transformation of carotene under these conditions, its function as an antioxidant must be extended to include the capacity to prevent the shift in electrons, if such it be, which attends the thermal or photoelectric change of the unstable to the colorless and more stable form of carotene."

Table 1 also reveals another interesting phenomenon; namely, that the exent of vitamin A inactivation produced by heated fats depends upon the temperature to which the fat is subjected. Experiments number 18, 19, 20, and 21 show that record of performance lard heated at or below 80° C. fails to develop the characteristic substances responsible for vitamin A destruction. The question arises whether the same effects of temperature apply to all fats; unfortunately an answer cannot be given from the data at hand. Furthermore, although heated animal fats 5 and 10 inactivated butterfat (experiments 6 and 11, table 1), they did not do so when the ration was mixed daily.

The stored animal fats (experiments 14, 15, and 16) did not act alike. The unheated commercial storage animal fat 15 (purchased on the market) caused destruction of vitamin A when mixed with butterfat; whereas, the other two unheated stored animal fats did not do this. Animal fat 15 was rancid organoleptically while animal fats 13 and 14 were not. It would appear, therefore, that rancidity is in some manner correlated with the capacity of a fat to destroy vitamin A when mixed with butterfat; in other words, from the standpoint of effect upon vitamin A, the development of rancidity within a fat results in transformations analogous to those produced by heating.

It will be observed from the data in table 6 that animal fat 5, vegetable fat 2, and animal fat 11 did not cause destruction of vitamin A in the cookies containing egg yolk. Experiment A reveals that in the first series of butter cookies vegetable fat 2 prevented destruction of vitamin A activity of the butter contained in the cookies; whereas, animal fat 11 and animal fat 5 did not do this. The data also show that vitamin A was destroyed when butter constituted the only fat in the cookies (lot VI, experiment A).

Experiment B shows that vitamin A of butter was not destroyed in the cookies containing either vegetable fat 2 or animal fat 11, but it was destroyed in the cookies containing animal fat 5 and in the cookies containing butter alone. The same batches of animal fat 5 and animal fat 11 were employed in the butter cookies, experiments A and B, table 6; but since experiment B was performed several months after experiment A the animal fat 5 and animal fat 11 employed in experiment B were older samples. The samples of animal fat 5 and animal fat 11 were stored in a cooler at approximately 0° C. Two different samples of vegetable fat 2 were employed in the butter cookies in experiments A and B. The data indicate either that a chemical change had occurred in the animal fat 11 during

storage which so altered it that when incorporated with the cookies vitamin A destruction was prevented; or, what appears more probable, that the conditions involved in the mixing of the ingredients of the cookies and subsequent heating were not absolutely uniform and, therefore, different results were obtained. It is difficult to prepare two batches of baked products exactly alike even from the same mix, and it is just as difficult to know whether or not they are alike. However, the temperature of the ingredients when mixed was 24.5° C. ± 1° C. Mixing time, rolling, and thickness of the mix were kept constant. Baking temperature was constant at 170° C. for 11 minutes and, because extreme care was used to keep conditions constant, it is difficult to explain the different results obtained in experiments A and B.

The cookies remaining after experiment A was completed were stored for 10 months at a temperature of 26° C. and then fed to the animals. The results are given in experiment C, table 6. Even after this long storage period vegetable fat 2 prevented destruction of vitamin A in the cookies. Two new series of butter cookies were prepared carrying different samples of animal fat 5, vegetable fat 2, and animal fat 11 than were employed in the first two series of butter cookies. The results are recorded in table 6 (experiments D and E, lot VI to X, inclusive). Cookies of series D were kept at approximately 26° C., and cookies of series E were stored at a temperature from —6° C. to 10° C. In experiment D vitamin A was destroyed only in the vegetable fat 2 cookies; while in experiment E vitamin A was not destroyed in any of the cookies. It would appear from the results that storage temperature may play some part in the destruction of vitamin A in baked products.

Three samples of coconut oil (experiments 37, 38, 39) on heating failed to destroy vitamin A in butterfat; whereas, four other samples of coconut oil (experiments 40, 41, 42, 43) on heating caused destruction of vitamin A in butterfat. Now it is possible that some coconut oils contain certain antioxidants or substances of unknown function whose presence prevents the oil from developing the property of vitamin A destruction when subjected to heat. Substances that prevent decomposition of fats have been recognized by Anderegg and Nelson (10); it is probable that substances of like function may play an important role in the preservation of vitamin A in the baking process or in the butterfat when mixed with certain heated fats. In an article entitled "Milk Powders as Food II," Anderegg and Nelson state: "During this work a marked difference was observed between the nutritive value of whole milk powder and skimmed milk powder. This difference cannot be attributed solely to a difference in the fat content; for when skimmed milk powder was suitably supplemented so that the resulting diet conformed in chemical composition to one of whole milk powder, on which very good results concerning reproduction were obtained, the outcome on the skimmed milk powder diet was entirely different. When cod liver oil is incorporated in skimmed milk powder diets, as a source of fat soluble vitamins, it undergoes decomposition giving rise to products strongly suggestive of acrolein. Other highly desicated materials also induce this decomposition. Addition of ethyl alcohol, wheat oil, or water to such mixtures exerts a protective action. Skimmed milk powder diets, upon which rats are sterile, were so changed by the addition of water and administering the cod liver oil separately that fourth generation young have now been obtained." The similarity,

from a chemical standpoint, between the work of Anderegg and Nelson and some of the results of this paper are striking.

## SUMMARY

1. Most of the heated fats that were studied caused destruction of vitamin A in butterfat.

2. Some heated oleomargarines and some heated coconut oils destroyed vitamin A; whereas, others did not.

3. The data seem to indicate that peroxides in heated fat are not the only factors concerned in the destruction of vitamin A.

4. Heating fat at or below 80° C. did not develop the characteristic change in the fat which causes inactivation of vitamin A.

5. Rancid fats appear to cause destruction of vitamin A.

6. The effects of the addition of fats to baked products on the vitamin A of the products will depend on the nature of the substances used as a source of vitamin A in the baked products, possibly upon the nature of the fats added, and upon certain unknown factors involved in the baking process.

## LITERATURE CITED

1. FRIDERICA, L. S.
1924-25. Inactivating action of some fats on vitamin A in other fats. J. Biol. Chem., 62:471-485.

2. DRUMMOND, J. C.
1919. Researches on the fat soluble accessory substance. I. Observations upon its nature and properties. Biochem J., 13:81-94.

3. POWICK, WILMER C.
1925. Inactivation of vitamin A by rancid fat. J. Agr. Res., 31:1017-1026.

4. MATTILL, H. A.
1927. The oxidative destruction of vitamins A and E. J. A. M. A., 89:1505-1508.

5. WHIPPLE, DOROTHY V.
1936. The destruction of vitamin A by rancid cod liver oil. Oil and Soap, 13:231-232.

6. NELSON, V. E., P. MABEL NELSON, and BELLE LOWE
1933 to 1938. Annual reports on agricultural research. Agricultural Experiment Station, Iowa State College.

7. LEASE, E. J., JANE G. LEASE, JANET WEBER, and H. STEENBOCK
1938. Destruction of vitamin A by rancid fats. J. Nutrition, 16:571-583.

8. MCCOLLUM, E. V., and MARGUERITE DAVIS
1914. Observations on the isolation of the substance in butterfat which exerts a stimulating influence on growth. J. Biol. Chem., 19:245-250.

9. OLCOVICH, H. S., and H. A. MATTILL
1931. The unsaponifiable lipids of lettuce. J. Biol. Chem., 91:105-117.

10. ANDEREGG, L. T., and V. E. NELSON
1926. Milk powders as food. II. Observations on the existence of vitamin E. J. Ind. and Eng. Chem., 18:620-627.

# THIAMIN EFFECTS IN BACTERIAL METABOLISM

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A review is given in this report of some of the work done in our laboratory during the past year involving the physiological effect of thiamin or vitamin $B_1$ upon the living cell. Its specific function in the glucose metabolism of living tissue was first reported by R. A. Peters (1) who was able to demonstrate that pyruvic acid in animal tissue arising from the decomposition of carbohydrates cannot be oxidized in the absence of thiamin. Similar results have been obtained by us employing bacterial cells grown in thiamin deficient media (Cf. references 6, 7, 8, 9, 10). The cells used in this work were grown in the basal medium of Tatum, Wood, and Peterson (2) in the absence of any added thiamin. Their ability to oxidize pyruvate under anaerobic conditions was tested in simple Warburg manometers. The results are shown in table 1.

TABLE 1. *The oxidation of pyruvic acid by Propionibacterium*

| | Cell suspension | | |
|---|---|---|---|
| | *Propionibacterium pentosaceum* | *Propionibacterium peterssonii* | *Lactobacillus mannitopoeus* |
| No activator ..... | 85 | 5 | 23 |
| Two micrograms thiamin added to suspension ..... | 480 | 120 | 90 |

Note: Results in $mm^3$ $CO_2$ evolved in 3 hours from sodium pyruvate.

The catalytic action of thiamin in the anaerobic pyruvate metabolism of the propionic acid bacteria and the single species of heterolactic acid bacteria is clearly brought out in table 1. In the absence of thiamin, *Propionibacterium peterssonii* is unable to oxidize pyruvate; on the addition of 2 micrograms of the vitamin to 2 cc. of the cell suspension, pyruvate is attacked. Similar results are obtained with suspensions of *Propionibacterium pentosaceum* and *Lactobacillus mannitopoeus*. *Escherichia coli* and *Aerobacter indologenes*, grown in a thiamin-free medium, do not show an increase in pyruvate metabolism on the addition of thiamin. They are capable of synthesizing the vitamin probably as rapidly as it is required. Sunderlin and Werkman (3) in 1928 reported the synthesis of vitamin B by *E. coli*.

Diphosphothiamin or cocarboxylase is essential for the decarboxylation of pyruvic acid by yeast. Dried yeast can be freed of its cocarboxylase by washing with alkaline phosphate. It now no longer can break down pyruvic acid except on the addition of cocarboxylase; free thiamin is ineffective. Since we had found that free thiamin stimulates the propionic and lactic acid bacteria, it was of interest to determine whether or not the thiamin was esterified before it catalyzed the breakdown of pyruvic acid. Present evidence indicates that such is the case.

We have found that *P. pentosaceum* is capable of synthesizing cocarboxylase. If *P. pentosaceum* is transferred from a yeast extract-peptone medium to a basal medium free of thiamin, the thiamin content of the cells is almost completely depleted by the third transfer on the basal medium. If to these cells, after thorough washing, free thiamin and phosphate buffer are added, a synthesis of cocarboxylase occurs. This is evidenced by data presented in table 2.

TABLE 2. *Synthesis of cocarboxylase by depleted cells of Propionibacterium*

| Activator | Supernate 1 | Supernate 2 | 2.3 micrograms cocarboxylase | Control |
|---|---|---|---|---|
| $mm^3$ $CO_2$ in 30 min. ..... | 324 | 28 | 236 | 21 |

Note: Test cells—yeast washed free of cocarboxylase.
Substrate—sodium pyruvate.

Supernate 1 had been obtained from a mixture of thiamin depleted cells, thiamin and phosphate buffer after 4 hours' incubation at 30° C. Supernate 2 was an identical mixture which was not incubated, but boiled immediately after mixing. As is readily seen, yeast washed free of cocarboxylase is reactivated by that synthesized by the bacteria, leaving little doubt that *P. pentosaceum* is capable of synthesizing cocarboxylase from thiamin and phosphate buffer.

Further evidence that thiamin is converted into cocarboxylase by thiamin deficient propionic acid bacteria may be obtained by a comparison of the stimulating action of free thiamin and crystalline cocarboxylase in the anaerobic pyruvate metabolism of these cells. Such data are presented in table 3.

TABLE 3. *Comparison of vitamin $B_1$ and cocarboxylase as stimulants in the anaerobic pyruvate metabolism of P. pentosaceum grown in vitamin $B_1$ deficient media*

| Cup | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| | | 0.25 microgram vitamin $B_1$ | | 0.34 microgram cocarboxylase | |
| Activator | None | Main vessel | Side cup | Main vessel | Side cup |
| Interval min. 0-30 ..... | 28 | 95 | 51 | 95 | 60 |
| 30-60 ..... | 8 | 64 | 35 | 59 | 51 |
| 60-90 ..... | 7 | 54 | 32 | 53 | 50 |
| 90-120..... | 7 | 55 | 41 | 56 | 52 |
| 120-150..... | 6 | 42 | 37 | 44 | 50 |
| 150-180..... | 5 | 46 | 41 | 46 | 47 |

Note: Results in $mm^3$ corrected for endogenous $CO_2$.

In cups 2 and 4, containing equimolar concentrations of thiamin and cocarboxylase, the cells had been incubated with the activators 45 minutes before the addition of pyruvic acid. The rates of gas production during the

intervals recorded are similar. In cups 3 and 5, equimolar quantities of the two activators were added to the cells at the time of the addition of pyruvic acid—no initial period of incubation of the cells and activators was permitted. Cup 5 containing added cocarboxylase attained the rates of the others after a 30 minute lag. Cup 3 containing the free thiamin required from 90 to 120 minutes to attain the rates of the others. It is reasonable to assume that this time is required for the synthesis of the diphosphoric ester of thiamin. In any case, if no previous incubation with the bacterial cells is permitted, cocarboxylase is a more effective stimulant than is free thiamin.

Work by Robbins (4) of the Missouri Botanical Gardens and by others has shown that some fungi are capable of synthesizing either or both of the two fractions[1] of the thiamin molecule to satisfy their growth requirements. We have tested the ability of *P. pentosaceum* to utilize either fraction separately or both together as a substitute for thiamin. It has been found that cells of *P. pentosaceum* cannot use the individual fractions as a substitute for the vitamin even when both are present—apparently these bacteria are unable to couple the two fractions to form the complete vitamin.

In reporting on the nutrition of the propionic acid bacteria, Wood, Andersen, and Werkman (5) have shown that one of them, *P. pentosaceum* can be trained to grow as well in the absence of thiamin as in its presence. Their data indicate that adaptation occurred after continuous serial transfer in an ammonium sulfate medium containing only ether extract of Difco yeast extract as a stimulant; the organism gradually acquired the ability to dispense with the vitamin. These investigators did not determine whether the organism trained to dispense with the vitamin acquires the ability to synthesize it, or whether its metabolism is diverted so that the vitamin is no longer required. We have found that the organism acquires the ability to synthesize thiamin.

TABLE 4. *Comparative yields of cells, their vitamin $B_1$ content and anaerobic pyruvate metabolism*

| | Yield of wet cells cc. | Vitamin $B_1$ content per gram dry cells micrograms | Anaerobic pyruvate metabolism $mm^3$ $CO_2$/hr. |
|---|---|---|---|
| "Trained" cells | 1.15 | 6.25 | 120.3 |
| "Untrained" cells | 0.50 | 0.40 | 15.2 |
| Ratio $\frac{\text{"trained"}}{\text{"untrained"}}$ | 2.3 | 15.6 | 7.9 |

Table 4 summarizes the results obtained in a series of experiments comparing cells of *P. pentosaceum* which had been carried three transfers ("untrained cells") and ten transfers ("trained cells") on a basal medium free of thiamin.

[1] Pyrimidine fraction employed was 2-methyl-5-bromomethyl-6-amino pyrimidine hydrobromide.
Thiazole fraction employed was 4-methyl-5-$\beta$-hydroxyethyl thiazole.

## LITERATURE CITED

1. PETERS, R. A.
1936. Pyruvic acid oxidation in brain. Biochem. J., 30: 2206.

2. TATUM, E. L., H. G. WOOD, and W. H. PETERSON
1936. Growth factors for bacteria. Biochem. J., 30: 1898.

3. SUNDERLIN, G., and C. H. WERKMAN
1928. Synthesis of vitamin B by microörganisms. J. Bact., 16: 17.

4. ROBBINS, W. J.
1938. Organisms requiring vitamin $B_1$. Proc. Nat. Acad. of Sciences U. S. A., 24: 53.

5. WOOD, H. G., A. A. ANDERSEN, and C. H. WERKMAN
1938. Nutrition of the propionic acid bacteria. J. Bact., 36: 201.

6. SILVERMAN, M., and C. H. WERKMAN
1938. Vitamin $B_1$ in bacterial metabolism. Proc. Soc. Exp. Biol. Med., 38: 823.

7. SILVERMAN, M., and C. H. WERKMAN
1939. Bacterial synthesis of cocarboxylase. Enzymologia, 5: 385.

8. SILVERMAN, M., and C. H. WERKMAN
1939. Bacterial synthesis of cocarboxylase. Proc. Soc. Exp. Biol. and Med., 40: 369.

9. SILVERMAN, M., and C. H. WERKMAN
1939. Function of vitamin $B_1$ in anaerobic bacterial metabolism. Iowa State Coll. J. of Science, 13: 107.

10. SILVERMAN, M., and C. H. WERKMAN
1939. Adaptation of the propionic acid bacteria to vitamin $B_1$ synthesis including a method of assay. J. Bact., 38: 25.

# ASYMPTOTIC SOLUTION OF A BOUNDARY VALUE PROBLEM[1]

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Many problems in the applications of mathematics lead to questions of the following kind: let $L^n(y)$ denote a differential expression—partial or ordinary—of order $n$, $\lambda$ a parameter, and $y = y(x, \lambda)$ the solution of a certain boundary value problem concerning an equation of the form

$$L^n(y) + \lambda L^m(y) = 0 \qquad (m < n). \tag{1}$$

The questions are then: first, whether—as seems plausible—$\lim_{\lambda \to \infty} y(x, \lambda)$ exists and is a solution of the equation $L^m = 0$; second, what boundary conditions this limit satisfies since $y(x, \lambda)$ satisfies $n$ boundary conditions while $L^m = 0$ is an equation of order $m < n$.[2]

The investigation of the relationship between the theories of viscous and ideal liquids (vanishing viscosity) offers famous examples for problems of this kind;[3] another example is furnished by the mathematical theory of the skin effect.[4]

In some former papers the present author dealt with certain linear and non-linear problems of the type mentioned above for the case $m = 0$.[5] It is the aim of the present note to give a simple example in which the method used in these papers can be applied also for the case $m = 1$.

THEOREM. *Let $y(x, \lambda)$ be the solution of the boundary value problem*

$$y'' + \lambda(y' - y) = \lambda f, \tag{1a}$$

$$y(0) = y(1) = 0 \tag{1b}$$

*where $\lambda$ is a positive parameter and the given function $f(x)$ is continuous in the interval $0 \leqq x \leqq 1$. Let $\eta(x)$ be the solution of the problem*

$$\eta' - \eta = f, \tag{2a}$$

$$\eta(1) = 0. \tag{2b}$$

[1] Presented to the American Mathematical Society, November 26, 1938.

[2] The important investigations of Birkhoff, Tamarkin, Langer, Trjitzinski, and other writers dealing with the asymptotic integration of ordinary differential equations lie in another direction. References concerning these investigations may, for instance, be found in W. J. Trjitzinski's paper, "Theory of linear differential equations containing a parameter," Acta Mathematica, 67:1-50.

[3] See C. W. Oseen, Hydrodynamik, Leipzig, 1927.

[4] For a treatment from a purely mathematical point of view, see E. Rothe, Zur Integralgleichung des Skineffekts, Journal f. riene u. angewandte Mathematik, 170 (1934), where other references concerning papers on the skin effect may be found.

[5] Über asymptotische Entwicklungen bei Randwertaufgaben elliptischer partieller Differentialgleichungen, Mathematische Annalen, 108 (no. of volume) (1933); Über asymptotische Entwicklungen bei Randwertaufgaben der Gleichung $\Delta\Delta u + \lambda u = \lambda^\kappa \psi$, Mathematische Annalen, 109 (1933); Über asymptotische Entwicklungen bei gewissen nichtlinearen Randwertaufgaben, Compositio Mathematica, 3 (1936).

Then

$$\lim_{\lambda\to\infty} y(x,\lambda)=\eta(x) \quad \text{for } 0<x\leqq 1. \tag{3}$$[6]

For the proof, we first state the following:

LEMMA I. Let $F(x,\lambda)$ be a given continuous function $(0\leqq x\leqq 1, 0\leqq\lambda<\infty)$ and $M(\lambda)=\underset{0\leqq x\leqq 1}{Max}|F(x,\lambda)|$. The solution $v$ of

$$v''+\lambda(v'-v)=F \qquad v(0)=v(1)=0 \tag{4}$$

satisfies the inequality

$$|v(x,\lambda)|<\frac{5M(\lambda)}{\lambda} \tag{5}$$

for sufficiently large $\lambda$.

*Proof of Lemma I.* Putting

$$v=e^{-\frac{1}{2}\lambda x}z, \tag{6}$$

one gets from (4)

$$z''-\nu z=F\,e^{\frac{1}{2}\lambda x} \qquad z(0)=z(1)=0 \tag{7}$$

with

$$\nu=\frac{\lambda^2}{4}+\lambda. \tag{8}$$

The solution of (7) may be written in the form

$$z(x)=\int_0^1 g(x,\xi,\lambda)\;F(\xi,\lambda)\;e^{\frac{1}{2}\lambda\xi}\,d\xi \tag{9}$$

where $g$ is the Green's function belonging to (7), namely,

$$g(x,\xi,\lambda)=\frac{-1}{\sqrt{\nu}\sinh\sqrt{\nu}}\begin{cases}\sinh\sqrt{\nu}(1-\xi)\sinh\sqrt{\nu}\,x & \text{for } \xi\geqq x\\ \sinh\sqrt{\nu}(1-x)\sinh\sqrt{\nu}\,\xi & \text{for } \xi<x.\end{cases} \tag{9a}$$

We see from (9) and (6) that

$$|v(x)|\leqq M(\lambda)\,I(x) \tag{10}$$

if

$$I(x)=-\int_0^1 g(x,\xi,\lambda)\;e^{-\frac{1}{2}\lambda(x-\xi)}\,d\xi,\quad M(\lambda)=\underset{0\leqq x\leqq 1}{Max}|F(x,\lambda)|$$

On the other hand, elementary integration furnishes

$$\lambda I(x)=1-\frac{e^{-(\sqrt{\nu}+\frac{1}{2}\lambda)x}(1-e^{-2\sqrt{\nu}(1-x)})+e^{-(\sqrt{\nu}-\frac{1}{2}\lambda)(1-x)}(1-e^{-2\sqrt{\nu}x})}{1-e^{-2\sqrt{\nu}}}.$$

[6] As $\lambda\to\infty$, the solution of $y''-\lambda(y'+y)=\lambda f$ for which $y(0)=y(1)=0$ approaches, in $0\leqq x<1$, that solution of $-(\eta'+\eta)=f$ which is zero for $x=0$. This case may be reduced to the one treated in the above theorem by substituting $1-x$ for $x$.

Therefore,

$$\lambda I \leqq 1 + \frac{2}{1 - e^{-2\sqrt{\nu}}}$$

which, on account of (10), proves (5).

LEMMA II. Let $h(x)$ be defined and continuous in $(0,1)$, $u$ the solution of

$$u'' + \lambda(u' - u) = \lambda h \text{ with } u(0) = u(1) = 0, \tag{11}$$

and $w$ the solution of

$$w' - w = h, \text{ with } w(1) = 0. \tag{12}$$

If then $h$ is such that

$$\int_0^1 h(\xi)\, e^{-\xi}\, d\xi = 0, \tag{13}$$

then

$$\lim_{\lambda \to \infty} u(x) = w(x) \tag{14}$$

uniformly in $0 \leqq x \leqq 1$.

*Proof of Lemma II.* As is easily seen, the additional condition (13) together with (12) implies $w(0) = 0$. Hence, it follows from (11) and (12) that $v = u - w$ is the solution of problem (4) with $F = -w''$, and (14) is a consequence of (5).

We turn now to the proof of equation (3) of the theorem. If $\varepsilon$ is an arbitrary positive number less than 1, it will be sufficient to prove (3) for $\varepsilon \leqq x \leqq 1$. For this purpose we define a function $h$ by

$$\begin{cases} h(x) = f(x) & \text{for } \tfrac{1}{2}\varepsilon \leqq x \leqq 1 \\ h(x) = [f(\tfrac{1}{2}\varepsilon) + (x - \tfrac{1}{2}\varepsilon)\, a]\, e^{x - \frac{1}{2}\varepsilon} & \text{for } 0 \leqq x < \tfrac{1}{2}\varepsilon \end{cases} \tag{15}$$

where

$$a = \frac{4}{\varepsilon} f(\tfrac{1}{2}\varepsilon) + \frac{8}{\varepsilon^2} e^{\frac{1}{2}\varepsilon} \int_{\frac{1}{2}\varepsilon}^1 f(\xi)\, e^{-\xi}\, d\xi.$$

As may be easily verified, $h$ satisfies the hypothesis (13) of Lemma II, so that (14) is true. On the other hand, it follows from (1) and (11) that $v = y - u$ is the solution of (4) with $F = \lambda(f - h)$. Hence it follows from (9), (6), and the definition of $h$ that

$$y - \mathrm{u} = \lambda \int_0^{\frac{1}{2}\varepsilon} [f(\xi) - h(\xi)]\, e^{-\frac{1}{2}\lambda(x - \xi)}\, g(x, \xi, \lambda)\, d\xi.$$

Therefore

$$|y - u| \leqq -\lambda \int_0^{\frac{1}{2}\varepsilon} e^{-\frac{1}{2}\lambda(x-\xi)} g(x, \xi, \lambda)\, d\xi \cdot \{Max|f| + Max|h|\} \tag{16}$$

Using the fact that $x > \xi$, one obtains from the definition (9a) of $g$

$$(17)\quad -\lambda\int_0^{\infty} e^{-i\lambda(x-\xi)}g(x,\xi,\lambda)\,d\xi = \frac{\lambda}{\sqrt{\nu}}\int_0^{\infty} e^{-i\lambda(x-\xi)}\frac{\sinh\sqrt{\nu}(1-x)\sinh\sqrt{\nu}\xi\,d\xi}{\sinh\sqrt{\nu}}$$

Since $x-\xi \geqq \frac{1}{2}\varepsilon$, we see from (17) and (16) that $\lim_{\lambda\to\infty}(y-u)=0$. Hence, on account of (14), $\lim_{\lambda\to\infty} y = w$, where $w$ is the solution of the problem (11). But, on account of the definition of $h$, the solution of problem (11) is identical with the solution $\eta$ of problem (2a), (2b) for $\frac{1}{2}\varepsilon \leqq x \leqq 1$. This proves equation (3) for $\varepsilon \leqq x \leqq 1$.

# THE THEORY OF TOPOLOGICAL ORDER IN SOME LINEAR TOPOLOGICAL SPACES[1]

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## 1. INTRODUCTION

Let $E$ be a space of the following properties:

1. $E$ is topological, that is, to each subset $A \subset E$ exists a subset $\overline{A} \subset E$, the closure of $A$, with the following properties. (a) $\overline{(\overline{A})} = \overline{A}$; (b) $\overline{A+B} = \overline{A} + \overline{B}$; (c) if $A$ contains not more than one point, then $\overline{A} = A$.[2]
2. $E$ is a linear vector space.[3] The operations implied in the definition of a linear vector space (addition of points and multiplication of a point with a real number) are supposed to be continuous.
3. There exists at least one convex[4] neighborhood $U$ of the zero point $0$ of $E$ which is "bounded," that is, has the following property: if $\alpha_1, \alpha_2 \ldots$ is a sequence of real numbers converging to zero and $\mathfrak{x}_1, \mathfrak{x}_2, \ldots$ an arbitrary sequence of points of $U$, then $\lim_{n\to\infty} \alpha_n x_n = 0$.[5]

Throughout this paper we denote by $V$ a closed convex bounded set in $E$ containing at least one interior point and by $S$ its boundary. Let $m < M$ be two positive numbers, $\lambda(\mathfrak{x})$ a continuous function defined for $\mathfrak{x} \subset S$, and $\mathfrak{F}(\mathfrak{x})$ a completely continuous representation of $S$;[6] the image is supposed to lie in $E$. We consider the representation

$$\mathfrak{y} = \mathfrak{f}(\mathfrak{x}) = \lambda(\mathfrak{x})\mathfrak{x} + \mathfrak{F}(\mathfrak{x}) \qquad (0 < m \leqq \lambda(\mathfrak{x}) \leqq M). \tag{1.1}$$

In generalization and extension of a former paper,[7] it is the aim of the present paper to develop the theory of the order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ of a point $\mathfrak{y}_0 \subset E$ not lying on the image $\mathfrak{f}(S)$ of $S$.[8]

In Section 2 a number of properties of the order which are well

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[1] Parts of this paper have been presented to the American Mathematical Society December 28, 1937, and April 8, 1938.

[2] The definition adopted here is the one used in [1] and [5]. (The numbers in brackets refer to the list of references at the end of this paper.) In the terminology used in [2], p. 37 and 60, this is a topological $T_1$ space.

[3] [3], p. 26.

[4] A subset $V$ of $E$ is said to be convex if $\mathfrak{x}_1 \subset V$, $\mathfrak{x}_2 \subset V$ implies $t\mathfrak{x}_1 + (1-t)\mathfrak{x}_2 \subset V$ for $0 \leqq t \leqq 1$. This set of points is called the segment connecting $\mathfrak{x}_1$ and $\mathfrak{x}_2$.

[5] This definition is due to Kolmogoroff [5].

[6] A representation is said to be completely continuous if it is continuous and if the image of each bounded set is compact.

[7] [10].

[8] For the theory of the order in $n$-dimensional spaces see for instance [2], Chapter XII.

known for $n$-dimensional spaces are proved in the special case that $E$ is a normed space.[9]

According to a theorem due to Kolmogoroff[10] each space $E$ can be normed. For the definition of the order in the general case it is, therefore, sufficient to show that the order is independent of the special norm which has been introduced. This is done in Section 3.

In Section 4 we deal with a space $E$ which is normed and strictly convex.[11] In this case it is proved that the equality of the two orders of a point $\mathfrak{y}_0 \subset E$ with respect to two representations of the form (1.1) is not only necessary but also sufficient for the homotopy of the two representations in $E - \mathfrak{y}_0$. Furthermore, the notion of the degree of the representation of an $S$ into an $S_1$ is introduced. It is proved that this degree is zero if not all points of $S_1$ are image points. Also, it is shown from the theorem on homotopy just mentioned that the equality of the two degrees of two representations is necessary and sufficient for the homotopy of the two representations in $S_1$.

## 2. NORMED SPACES

Throughout this section $E$ is supposed to be a normed linear vector space.[12] An $n$-dimensional linear subspace of $E$ is denoted by $E^n$. The representation

$$\mathfrak{H}(\mathfrak{x}) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}), \tag{2.1}$$

defined in a certain subset of $E$ is called a "layer representation" (with respect to $E^n$)[13] if $\mathfrak{S}(\mathfrak{x}) \subset E^n$ and if, moreover, $\mathfrak{S}$ is continuous. Evidently a layer representation with respect to $E^n$ is also a layer representation with respect to any $E^m$ which contains $E^n$.

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[9] $E$ is said to be normed if to each element $\mathfrak{x} \subset E$ is associated a non-negative number $||\mathfrak{x}||$, the norm of $\mathfrak{x}$, of the following properties: $||\mathfrak{x}|| = 0$ only for the zero element of $E$; $||\lambda\mathfrak{x}|| = |\lambda|\,||\mathfrak{x}||$ for any real number $\lambda$; $||\mathfrak{x} + \mathfrak{y}|| \leqq ||\mathfrak{x}|| + ||\mathfrak{y}||$. For two elements $\mathfrak{x}$ and $\mathfrak{y}$ the distance is defined as the norm of the difference. Cf. [3], p. 53. Section 2 is closely related to § 2 of paper [10]; all developments, however, are given in such a way that the present paper may be understood without knowledge of [10] and references to this paper are only made for the proof of some special facts. For readers familiar with [10] I indicate some of the differences between Section 2 of the present paper and § 2 of [10]: the use of boundaries of convex sets instead of spheres, which was necessary in view of Section 3; the introduction of representations of the form (1.1), whereas in [10]—as well as in the fundamental paper [7]—only representations with $\lambda(\mathfrak{x}) \equiv 1$ have been considered (this generalization makes the use of central projection possible); the change in the definition of homotopy (cf. Definitions 4, 5, and Lemma 3 of the present paper.).

[10] [5].

[11] According to a definition due to J. A. Clarkson [4], p. 404, a normed space is called strictly convex if, in case the elements $\mathfrak{x}$ and $\mathfrak{y}$ are both different from the zero element, the equality sign in the inequality of Footnote 9 holds then, and only then, when, for a suitable positive number $c$, $\mathfrak{x} = c\mathfrak{y}$.

[12] Evidently any normed linear vector space satisfies Conditions 1-3 of the introduction if the closure is defined in terms of the distance in the usual way.

[13] Called "transformation dégénérée" in [6], p. 141, and "Schichtenabbildung" in [10], § 1, 7.

DEFINITION 1. *Let $V$ be a convex bounded set in $E$ containing at least one interior point, $S$ its boundary, $\mathfrak{s}(\mathfrak{x})$ a layer representation defined on $S$, and $\mathfrak{y}_0$ a point of $E$ not lying on $\mathfrak{s}(S)$. Let $E^n$ be a linear subspace of $E$ possessing the following properties:* (a) *$\mathfrak{s}$ is a layer representation with respect to $E^n$;* (b) *$E^n$ contains at least one interior point of $V$;* (c) *$E^n$ contains $\mathfrak{y}_0$. Let $V^n$ be the intersection of $V$ and $E^n$, and $S^{n-1}$ its boundary. $S^{n-1}$ is then the intersection of $E^n$ and $S$. The representation $\mathfrak{s}^n(x)$ defined by $\mathfrak{s}^n(\mathfrak{x}) = \mathfrak{s}(\mathfrak{x})$ for $\mathfrak{x} \subset S^{n-1}$ is then a continuous representation of $S^{n-1}$ in the space $E^n$ since $\mathfrak{s}$ is a layer representation with respect to $E^n$. As $\mathfrak{y}_0$ does not lie on $\mathfrak{s}^n(S^{n-1})$, the order $u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0)$ of $\mathfrak{y}_0$ with respect to $\mathfrak{s}^n(S^{n-1})$ is well defined according to the definition given in the topology of $n$-dimensional spaces. It can be proved that this order $u$ is the same for all linear subspaces $E^n$ having the properties* (a), (b), *and* (c).[14] *Therefore, it is legitimate to define the order $u(\mathfrak{s}, S, \mathfrak{y}_0)$ of $\mathfrak{y}_0$ with respect to $\mathfrak{s}(S)$ by the equation $u(\mathfrak{s}, S, \mathfrak{y}_0) = u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0)$.*

DEFINITION 2. *Let $\mathfrak{f}(\mathfrak{x})$ be a representation* (1.1) *defined on $S$ with $\lambda \equiv 1$. Let $\mathfrak{y}_0$ be a point not lying on $\mathfrak{f}(S)$ and $\varepsilon$ its positive distance from $\mathfrak{f}(S)$. Let $\mathfrak{s}(\mathfrak{x})$ be a layer representation defined on $S$ for which*

$$||\mathfrak{f}(\mathfrak{x}) - \mathfrak{s}(\mathfrak{x})|| < \varepsilon \qquad (\mathfrak{x} \subset S) \tag{2.2}$$

*holds*[15] *so that $\mathfrak{y}_0$ is different from $\mathfrak{s}(S)$. It can be proved that the order $u(\mathfrak{s}, S, \mathfrak{y}_0)$ given by Definition 1 is the same for all layer representations which satisfy* (2.2).[16] *By definition, we put $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{s}, S, \mathfrak{y}_0)$ and call this number the order of $\mathfrak{y}_0$ with respect to the image $\mathfrak{f}(S)$ of $S$.*

DEFINITION 3. *In order to define the order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ of a point $\mathfrak{y}_0$ not lying on $\mathfrak{f}(S)$ for a general representation* (1.1), *we put*

$$\mathfrak{f}(\mathfrak{x}, \mathfrak{y}_0) = \mathfrak{f}_0(\mathfrak{x}) = \mathfrak{y}_0 + \frac{1}{\lambda}[\mathfrak{f}(\mathfrak{x}) - \mathfrak{y}_0] = \mathfrak{x} + \mathfrak{F}_0(\mathfrak{x}) \tag{2.3}$$

*where*

$$\mathfrak{F}_0(\mathfrak{x}) = \mathfrak{y}_0\left(1 - \frac{1}{\lambda}\right) + \frac{1}{\lambda}\mathfrak{F}(\mathfrak{x}). \tag{2.4}$$

*The assumption that $\mathfrak{y}_0$ is not on $\mathfrak{f}(S)$ implies that $\mathfrak{y}_0$ is not on $\mathfrak{f}_0(S)$. Therefore, $u(\mathfrak{f}_0, S, \mathfrak{y}_0)$ is well defined according to Definition 2. By definition, we put $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{f}_0, S, \mathfrak{y}_0)$.*

THEOREM 1. *The order $u(\mathfrak{f}, S, \mathfrak{y}_0)$, as defined in Definition 3, remains constant when $\mathfrak{y}_0$ varies continuously without lying on $\mathfrak{f}(S)$.*

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[14] We omit the proof since it is essentially the same as the one given in [10], § 2, Hilfssatz 2, in the special case that $S$ is a sphere. (A sphere of center $\mathfrak{o}$ and radius $r$ is defined as the set of all points $\mathfrak{x}$ for which $||\mathfrak{x}|| = r$; the set of all points $\mathfrak{x}$ with $||\mathfrak{x}|| \leqq r$ is called a "full" sphere.)

[15] For the proof of the existence of such a layer representation, see [7], § 1, 7, or [10], p. 51.

[16] We omit the proof which is essentially the same as the one given in [10], § 2, Hilfssatz 4.

Before proving this theorem we state the following two lemmas:

LEMMA 1. *For each $t$ of the interval $0 \leqq t \leqq 1$, let $\mathfrak{s}(\mathfrak{x}, t)$ be a layer representation with respect to the linear subspace $E^n$, which is supposed to be independent of $t$. $\mathfrak{s}(\mathfrak{x}, t)$ is supposed to be continuous in $(\mathfrak{x}, t)$ and not to contain $\mathfrak{y}_0$. The order $u(\mathfrak{s}, S, \mathfrak{y}_0)$ is then independent of $t$.*

We omit the proof, which is nearly the same as the one given in [10] (Hilfssatz 3) in the special case of a sphere.

LEMMA 2. *Let $\mathfrak{s}$ be a layer representation defined on $S$, and $\mathfrak{y}_0$ a point not lying on $\mathfrak{s}(S)$. Denote by $\varepsilon$ the positive distance between $\mathfrak{y}_0$ and $\mathfrak{s}(S)$. Then*

$$u(\mathfrak{s}, S, \mathfrak{y}) = u(\mathfrak{s}, S, \mathfrak{y}_0) \quad \text{for } ||\mathfrak{y} - \mathfrak{y}_0|| < \varepsilon. \tag{2.5}$$

The proof follows immediately from the well known corresponding property of the order in $n$-dimensional spaces if one considers an $E^n$ possessing the properties (a), (b), (c) referred to in Definition 1, and, moreover, containing the point $\mathfrak{y}$ for which (2.5) is to be proved.

*Proof of Theorem* 1. If $\mathfrak{y}_0$ is a point not lying on $\mathfrak{f}(S)$, $\varepsilon$ its distance from $\mathfrak{f}(S)$, and $m$, $M$ the positive numbers of (1.1), it will be sufficient to prove

$$u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{f}, S, \mathfrak{y}_1) \tag{2.6}$$

for an arbitrary point $\mathfrak{y}_1$ for which the inequality

$$||\mathfrak{y}_1 - \mathfrak{y}_0|| < \frac{\varepsilon m}{5M_1(1+m)} \qquad [M_1 = Max(M, 1)] \tag{2.7}$$

holds. On account of Definition 3, Equation (2.6) is equivalent to

$$u(\mathfrak{f}_0, S, \mathfrak{y}_0) = u(\mathfrak{f}_1, S, \mathfrak{y}_1) \tag{2.8}$$

if

$$\mathfrak{f}_0 = \mathfrak{y}_0 + \frac{1}{\lambda}(\mathfrak{f} - \mathfrak{y}_0) = \mathfrak{x} + \mathfrak{y}_0\left(1 - \frac{1}{\lambda}\right) + \frac{1}{\lambda}\mathfrak{F}, \tag{2.9}$$

$$\mathfrak{f}_1 = \mathfrak{y}_1 + \frac{1}{\lambda}(\mathfrak{f} - \mathfrak{y}_1) = \mathfrak{x} + \mathfrak{y}_1\left(1 - \frac{1}{\lambda}\right) + \frac{1}{\lambda}\mathfrak{F}.$$

To prove (2.8), let $\mathfrak{s}_0(\mathfrak{x})$ and $\mathfrak{s}_1(\mathfrak{x})$ be two layer representations with[15]

$$||\mathfrak{f}_0(\mathfrak{x}) - \mathfrak{s}_0(\mathfrak{x})|| < \frac{\varepsilon}{5M_1}, \; ||\mathfrak{f}_1(\mathfrak{x}) - \mathfrak{s}_1(\mathfrak{x})|| < \frac{\varepsilon}{5M_1}. \tag{2.10}$$

We state that then

$$u(\mathfrak{f}_0, S, \mathfrak{y}_0) = u(\mathfrak{s}_0, S, \mathfrak{y}_0), \quad u(\mathfrak{f}_1, S, \mathfrak{y}_1) = u(\mathfrak{s}_1, S, \mathfrak{y}_1). \tag{2.11}$$

We have, in fact, from (2.10), (2.9), and the definition of $\varepsilon$

$$||\mathfrak{f}_0 - \mathfrak{s}_0|| < \frac{\varepsilon}{M_1} < ||\mathfrak{y}_0 - \mathfrak{f}_0(S)||$$

so that the first equation (2.11) follows from Definition 2. As to the second equation (2.11), we have from (2.9) and (2.7):

$$(2.12) \qquad ||\mathfrak{f}_0 - \mathfrak{f}_1|| \leqq ||\mathfrak{y}_0 - \mathfrak{y}_1|| \left(1 + \frac{1}{m}\right) \leqq \frac{\varepsilon}{5M_1},$$

and, by this, from (2.9), (2.7) and (2.10)

$$||\mathfrak{y}_1 - \mathfrak{f}_1|| \geqq ||\mathfrak{y}_0 - \mathfrak{f}_0|| - ||\mathfrak{f}_1 - \mathfrak{f}_0|| - ||\mathfrak{y}_0 - \mathfrak{y}_1|| \geqq \frac{\varepsilon}{M_1} - \frac{2\varepsilon}{5M_1} > ||\mathfrak{f}_1(\mathfrak{x}) - \mathfrak{s}_1(\mathfrak{x})||$$

which proves the second equation (2.11), again on account of Definition 2.

For the proof of (2.8), it will, on account of (2.11), be sufficient to prove

$$(2.13) \qquad u(\mathfrak{s}_0, S, \mathfrak{y}_0) = u(\mathfrak{s}_1, S, \mathfrak{y}_1).$$

We prove first

$$(2.14) \qquad u(\mathfrak{s}_1, S, \mathfrak{y}_1) = u(\mathfrak{s}_1, S, \mathfrak{y}_0).$$

This equation follows from Lemma 2 since, because of (2.9), (2.12), (2.10), and (2.7), the inequality

$$||\mathfrak{s}_1 - \mathfrak{y}_0|| \geqq ||\mathfrak{f}_0 - \mathfrak{y}_0|| - ||\mathfrak{f}_0 - \mathfrak{f}_1|| - ||\mathfrak{f}_1 - \mathfrak{s}_1||$$

$$> \frac{\varepsilon}{M_1} - \frac{\varepsilon}{5M_1} - \frac{\varepsilon}{5M_1} > ||\mathfrak{y}_1 - \mathfrak{y}_0||$$

holds. We now prove

$$(2.15) \qquad u(\mathfrak{s}_1, S, \mathfrak{y}_0) = u(\mathfrak{s}_0, S, \mathfrak{y}_0).$$

This equality follows from Lemma 1, for we have, because of (2.10) and (2.12),

$$||\mathfrak{s}_0 - \mathfrak{s}_1|| \leqq ||\mathfrak{s}_0 - \mathfrak{f}_0|| + ||\mathfrak{f}_0 - \mathfrak{f}_1|| + ||\mathfrak{f}_1 - \mathfrak{s}_1|| < \frac{3\varepsilon}{5M_1},$$

and, from this equation together with (2.9 and (2.10), the inequality

$$||\{\mathfrak{s}_0 + t(\mathfrak{s}_1 - \mathfrak{s}_0)\} - \mathfrak{y}_0|| \geqq ||\mathfrak{s}_0 - \mathfrak{y}_0|| - ||\mathfrak{s}_1 - \mathfrak{s}_0||$$

$$\geqq ||\mathfrak{f}_0 - \mathfrak{y}_0|| - ||\mathfrak{s}_0 - \mathfrak{f}_0|| - ||\mathfrak{s}_1 - \mathfrak{s}_0|| > \frac{\varepsilon}{M_1} - \frac{\varepsilon}{5M_1} - \frac{3\varepsilon}{5M_1} > 0$$

follows for $0 \leqq t \leqq 1$.

Since (2.13) follows from (2.14) and (2.15), our theorem is proved.

Our next aim is to prove the invariance of the order with respect to a continuous change of the representation $\mathfrak{f}$. Before doing so we have to define the exact meaning of a "continuous change."

DEFINITION 4. *Let $\lambda(\mathfrak{x}, t)$ be a function and $\mathfrak{F}(\mathfrak{x}, t)$ a representation, both defined for $0 \leqq t \leqq 1$ and for all $\mathfrak{x}$ of a certain bounded subset $A$ of $E$. We suppose:*

(a) *$\lambda$ and $\mathfrak{F}$ are continuous as functions of $(\mathfrak{x}, t)$;*

(b) *$M \geqq \lambda \geqq m > 0$, where the constants $m$ and $M$ are independent of $t$ and $\mathfrak{x}$,*

(c) *the set of all points* $\mathfrak{F}(\mathfrak{x}, t)$ *for* $\mathfrak{x} \subset A$ *and* $0 \leqq t \leqq 1$ *is compact.*

*Under these conditions we say, by definition, that the representation*

$$\mathfrak{f}(\mathfrak{x}, t) = \lambda(\mathfrak{x}, t)\,\mathfrak{x} + \mathfrak{F}(\mathfrak{x}, t) \tag{2.16}$$

*depends continuously on the parameter t. Moreover, if*

$$\mathfrak{f}_1(\mathfrak{x}) = \lambda_1(\mathfrak{x})\,\mathfrak{x} + \mathfrak{F}_1(\mathfrak{x}), \quad \mathfrak{f}_2(\mathfrak{x}) = \lambda_2(\mathfrak{x})\,\mathfrak{x} + \mathfrak{F}_2(\mathfrak{x}) \tag{2.17}$$

$$(0 < m \leqq \lambda_i(\mathfrak{x}) \leqq M;\ i = 1, 2)$$

*are two representations defined in A with continuous* $\lambda_i$*, and completely continuous* $\mathfrak{F}_i$*, we say, by definition, that* $\mathfrak{f}_1$ *and* $\mathfrak{f}_2$ *can be continuously transformed into each other or that they are homotopic if there exists a representation* $\mathfrak{f}(\mathfrak{x}, t)$ *depending, in the sense just defined, continuously on the parameter t, for which* $\mathfrak{f}(\mathfrak{x}, 0) = \mathfrak{f}_1(\mathfrak{x})$ *and* $\mathfrak{f}(\mathfrak{x}, 1) = \mathfrak{f}_2(\mathfrak{x})$*. If, moreover,* $\mathfrak{f}(\mathfrak{x}, t)$ *has no point in common with a certain subset E′ of E for all t of the interval* $0 \leqq t \leqq 1$*, we say that* $\mathfrak{f}_1$ *and* $\mathfrak{f}_2$ *are homotopic in* $E - E'$*.*

DEFINITION 5. *We say that the representation* (2.16) *is uniformly continuous (with respect to t) if in Definition* 4 *the conditions* (a) *and* (c) *are replaced by the following:*

(a′) *For each t,* $\lambda$ *is continuous and* $\mathfrak{F}$ *completely continuous in dependence on* $\mathfrak{x}$*.*

(c′) *As functions of t,* $\lambda$ *and* $\mathfrak{F}$ *are continuous, and uniformly so with respect to* $\mathfrak{x}$*, that is, to any given positive number* $\varepsilon$ *there exists a positive number* $\delta$ *which is independent of* $\mathfrak{x}$ *such that* $||t' - t''|| < \delta$ *implies*

$$|\lambda(\mathfrak{x}, t') - \lambda(\mathfrak{x}, t'')| < \varepsilon \text{ and } ||\mathfrak{F}(\mathfrak{x}, t') - \mathfrak{F}(\mathfrak{x}, t'')|| < \varepsilon$$

for $0 \leqq t \leqq 1$ and $\mathfrak{x} \subset A$.[17]

LEMMA 3. *A uniformly continuous representation (Definition* 5*) is continuous in the sense of Definition* 4*.*

For the proof, we refer to [9], p. 301, proof of Hilfssatz 2.

*Remark.* The converse of Lemma 3 is not true. An example of a representation satisfying Definition 4, but not the condition (c′) of Definition 5 is the following: let $E$ be the Hilbert space with co-ordinates $x_1, x_2, \ldots$, $A$ the sphere $\sum_{\nu=1}^{\infty} x_\nu^2 = 1$, and $\mathfrak{y} = \mathfrak{f}(\mathfrak{x}, t)$ the representation given by the equations $y_1 = x_1 + \sum_{\nu=1}^{\infty} x_\nu^2 t^\nu$, $y_2 = x_2$, $y_3 = x_3, \ldots$ $\quad (0 \leqq t \leqq 1)$

THEOREM 2. *Let* $\mathfrak{f} = \mathfrak{f}(\mathfrak{x}, t)$ *be a representation defined on the boundary S of the convex bounded set V and depending continuously on the parameter t for* $0 \leqq t \leqq 1$ *(Definition* 4*). Let* $\mathfrak{y}_0$ *be a point different from* $\mathfrak{f}(\mathfrak{x}, t)$ *for all* $\mathfrak{x} \subset S$ *and all t. Then the order* $u(\mathfrak{f}, S, \mathfrak{y}_0)$ *is independent of t.*

Before proving Theorem 2, we state the following:

[17] In the papers [7] and [10] the meaning of "continuous" is, in the terminology of the present paper, "uniformly continuous."

LEMMA 4. *Let* $\mathfrak{f}(\mathfrak{x}, t)$ *be a representation* (1.1), *with* $\lambda \equiv 1$, *defined in a certain subset* $A$ *of* $E$, *and depending continuously on* $t$ (*Definition* 4); *let* $\varepsilon$ *be a positive constant. Then there exist a linear subset* $E^n \subset E$ *which is independent of* $t$ *and a representation* $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}, t)$ *of the following properties:*

(a) $\mathfrak{s}$ *is a layer representation with respect to* $E^n$,

(b) $\mathfrak{s}$ *is continuous in* $(\mathfrak{x}, t)$,

(c) $||\mathfrak{s}(\mathfrak{x}, t) - \mathfrak{f}(\mathfrak{x}, t)|| < \varepsilon$.

*Proof.*[18] From Condition (c) in Definition 4 and the Heine-Borel theorem it follows that the set of points $\mathfrak{F}(\mathfrak{x}, t)$ $(\mathfrak{x} \subset A, 0 \leqq t \leqq 1)$ contains a finite number of points $\mathfrak{y}_1, \mathfrak{y}_2, \ldots, \mathfrak{y}_m$ such that, for each $\mathfrak{x}$ and $t$, the inequality

$$||\mathfrak{F}(\mathfrak{x}, t) - \mathfrak{y}_\alpha|| < \varepsilon$$

holds for at least one $\alpha$ $(1 \leqq \alpha \leqq m)$. Let us denote by $E^n$ the smallest linear subset of $E$ containing the points $\mathfrak{y}_1, \mathfrak{y}_2, \ldots, \mathfrak{y}_m$ and put

$$\mu_\nu(\mathfrak{y}) = \begin{cases} \varepsilon - ||\mathfrak{y} - \mathfrak{y}_\nu|| & \text{for } ||\mathfrak{y} - \mathfrak{y}_\nu|| \leqq \varepsilon \\ 0 & \text{for } ||\mathfrak{y} - \mathfrak{y}_\nu|| > \varepsilon. \end{cases}$$

If then

$$\mathfrak{S}(\mathfrak{x}, t) = \frac{\sum_{\nu=1}^{m} \mathfrak{y}_\nu \mu_\nu[\mathfrak{F}(\mathfrak{x}, t)]}{\sum_{\nu=1}^{m} \mu_\nu[\mathfrak{F}(\mathfrak{x}, t)]},$$

one sees easily that $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}, t)$ has the properties (a), (b), (c).

*Proof of Theorem* 2. Since $\mathfrak{y}_0$ is different from $\mathfrak{f}(\mathfrak{x}, t)$ it is, on account of the assumptions made concerning $\lambda$ and $\mathfrak{F}(\mathfrak{x}, t)$, easy to see that there exists a positive constant $\varepsilon'$ such that $||\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0|| > \varepsilon'$. Hence the representation $\mathfrak{f}_0$ defined in (2, 3) satisfies the inequality

$$||\mathfrak{f}_0(\mathfrak{x}, t) - \mathfrak{y}_0|| = \frac{1}{\lambda(\mathfrak{x}, t)} ||\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0|| > \frac{\varepsilon'}{M}$$

and, if $\varepsilon = \frac{\varepsilon'}{M}$, the hypotheses of Lemma 4. Now let $\mathfrak{s}$ be a layer representation of the properties described in Lemma 4. By Definition 2, we have $u(\mathfrak{f}_0, S, \mathfrak{y}_0) = u(\mathfrak{s}, S, \mathfrak{y}_0)$. Hence,

$$u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{s}, S, \mathfrak{y}_0) \tag{2.18}$$

since, by Definition 3, $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{f}_0, S, \mathfrak{y}_0)$. According to Lemma 1, $u(\mathfrak{s}, S, \mathfrak{y}_0)$ is independent of $t$; hence Theorem 2 follows from (2.18).

An immediate consequence of Theorem 2 is the following theorem

[18] C.f. the proof given by Leray-Schauder ([7], p. 51, second lemma) in the case of a representation not containing a parameter.

which, in analogy to the $n$-dimensional case,[19] will be referred to as the theorem of Poincaré-Bohl:

THEOREM 3 (Theorem of Poincaré-Bohl). *Let* $\mathfrak{f}(\mathfrak{x})$ *and* $\mathfrak{g}(\mathfrak{x})$ *be two representations defined on the boundary* $S$ *of a convex bounded set* $V$[21] *and having the same properties as the representation* (1.1). *Let* $\mathfrak{y}_0$ *be a point which, for each* $\mathfrak{x} \subset S$, *is not on the segment*[4] *connecting the points* $\mathfrak{f}(\mathfrak{x})$ *and* $\mathfrak{g}(\mathfrak{x})$. *Then* $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{g}, S, \mathfrak{y}_0)$.

For the proof, we have simply to apply Theorem 2 with

$$\mathfrak{f}(\mathfrak{x}, t) = (1-t)\mathfrak{f}(\mathfrak{x}) + t\mathfrak{g}(\mathfrak{x}) \qquad (0 \leqq t \leqq 1).$$

As is seen from the fact that

$$||(1-t)\mathfrak{f} + t\mathfrak{g} - \mathfrak{y}_0|| = ||\mathfrak{f} - \mathfrak{y}_0 + t(\mathfrak{g} - \mathfrak{f})|| \geqq ||\mathfrak{f} - \mathfrak{y}_0|| - ||\mathfrak{g} - \mathfrak{f}||$$

which holds for $0 \leqq t \leqq 1$, the following theorem is a special case of Theorem 3:

THEOREM 4 (Theorem of Rouché). *If for the two representations* $\mathfrak{f}$ *and* $\mathfrak{g}$ *having the same properties as the representation* (1.1) *the inequality*

$$||\mathfrak{f} - \mathfrak{g}|| < ||\mathfrak{f} - \mathfrak{y}_0||$$

holds, then $u(\mathfrak{f}, S, \mathfrak{y}_0) = u(\mathfrak{g}, S, \mathfrak{y}_0)$.[22]

Since $u(\mathfrak{x}, S, \mathfrak{y}_0) = 1$, the substitution of $\mathfrak{x}$ for $\mathfrak{f}(\mathfrak{x})$ in Theorem 4 immediately gives us

THEOREM 5. *If, for all* $\mathfrak{x} \subset S$, *the norm of the displacement* $\mathfrak{g} - \mathfrak{x}$ *is less than the distance between* $\mathfrak{y}_0$ *and* $\mathfrak{x}$, *then* $u(\mathfrak{g}, S, \mathfrak{y}_0) = 1$.

THEOREM 6. *Let* $\mathfrak{f}(\mathfrak{x}) = \lambda(\mathfrak{x})\mathfrak{x} + \mathfrak{F}(\mathfrak{x})$ $(0 < m \leqq \lambda(\mathfrak{x}) \leqq M)$ *be defined in all points* $\mathfrak{x}$ *of the convex bounded set* $V$ *(not only on its boundary* $S$*). As usual,* $\lambda$ *is supposed to be continuous, and* $\mathfrak{F}$ *completely continuous. Let* $\mathfrak{y}_0$ *be a point which is not image of a point of* $V$. *Then* $u(\mathfrak{f}, S, \mathfrak{y}_0) = 0$.

*Proof.* The theorem is true in case $\lambda \equiv 1$[23] and, therefore, for the representation (2.3) occurring in Definition 3. Since the assumption that $\mathfrak{y}_0$ is, for every $\mathfrak{x} \subset V$, different from $\mathfrak{f}(\mathfrak{x})$ implies that $\mathfrak{y}_0$ is different from $\mathfrak{f}_0(\mathfrak{x})$ also, we have $u(\mathfrak{f}_0, S, \mathfrak{y}_0) = 0$. According to Definition 3 our theorem is proved.

THEOREM 7. *Let* $\mathfrak{f}(\mathfrak{x}) = \lambda(\mathfrak{x})\mathfrak{x} + \mathfrak{F}(\mathfrak{x})$ *be a representation* (1.1) *defined on the boundary* $S$ *of* $V$, *and* $\mathfrak{y}_0$ *a point not lying on* $\mathfrak{f}(S)$. *If there exists a ray* $h$[24] *issuing from* $\mathfrak{y}_0$ *and not intersecting* $\mathfrak{f}(S)$, *then* $u(\mathfrak{f}, S, \mathfrak{y}_0) = 0$.

*Proof.* On account of Definition 3, it will be sufficient to prove the theorem for the representation $\mathfrak{f}_0(\mathfrak{x})$ defined in (2.3). Since $\mathfrak{y}_0$ is different

[19] See, for instance, [2], p. 459.

[20] See Footnote 4.

[21] We recall the convention of the introduction that $V$ contains an interior point.

[22] For the $n$-dimensional case, see [2], p. 459.

[23] Cf. [10], §2, Satz 3, where this fact is proved in case $S$ is a sphere.

[24] A ray $h$ issuing from $\mathfrak{y}_0$ and containing the point $\mathfrak{y}_1$ is the set of all points $\mathfrak{y}_0 + t(\mathfrak{y}_1 - \mathfrak{y}_0)$ for $0 \leqq t < \infty$. The "opposite" ray is the set of all points $\mathfrak{y}_0 - t(\mathfrak{y}_1 - \mathfrak{y}_0)$.

from $\mathfrak{f}_0(S)$, there exists a full sphere $V_0$ with center $y_0$ having no point in common with $\mathfrak{f}_0(S)$. We will now define a representation $\mathfrak{f}_0(\mathfrak{x})$ for all points $\mathfrak{x}$ of $V$ which on $S$ is identical with the given representation $\mathfrak{f}_0(\mathfrak{x})$. For this purpose, let $\mathfrak{x}_1$ be an arbitrary but fixed interior point of $V$. By definition, we put $\mathfrak{f}_0(\mathfrak{x}_1) = \mathfrak{y}_1$, where $\mathfrak{y}_1$ is an arbitrarily determined point in the interior of $V_0$, different from $\mathfrak{y}_0$ and on the ray opposite to $h$.[24] If $\mathfrak{x}$ is a point of $V$ which is different from $\mathfrak{x}_1$, let $\bar{\mathfrak{x}}$ be the intersection of $S$ and of the ray issuing from $\mathfrak{x}_1$ and containing $\mathfrak{x}$. If, then, $t$ is given by the equation $\mathfrak{x} = \mathfrak{x}_1 + t(\bar{\mathfrak{x}} - \mathfrak{x}_1)$, we define $\mathfrak{f}_0(\mathfrak{x}) = \mathfrak{y}_1 + t[\mathfrak{f}_0(\bar{\mathfrak{x}}) - \mathfrak{y}_1]$.[25] From this definition it is easy to see that $\mathfrak{y}_0$ is not an image of a point of $V$, since the ray issuing from $\mathfrak{y}_1$ and containing $\mathfrak{y}_0$ has no intersection with $\mathfrak{f}_0(S)$. Hence, Theorem 7 follows from Theorem 6.

An immediate consequence of Theorem 1 and Theorem 6 is

THEOREM 8. *Let $\mathfrak{f}$ be a representation satisfying the assumptions of Theorem 6. If $u(\mathfrak{f}, S, \mathfrak{y}_0)$ is defined and different from zero, then there exists a neighborhood of $\mathfrak{y}_0$ consisting entirely of points which are images of points of $V$.*

A consequence of Theorem 8 and Theorem 5 is

THEOREM 9 (Displacement Theorem). *Let $V, S, \mathfrak{f}$ have the same meaning as in Theorem 8 and $\mathfrak{y}_0$ be an interior point of $V$. If the norm of the displacement $\mathfrak{f}(\mathfrak{x}) - \mathfrak{x}$ is less than the distance between $\mathfrak{y}_0$ and $\mathfrak{x}$, that is, if $||\mathfrak{f}(\mathfrak{x}) - \mathfrak{x}|| < ||\mathfrak{y}_0 - \mathfrak{x}||$, then there exists a neighborhood of $\mathfrak{y}_0$ consisting entirely of image points.*

THEOREM 10 (Fixpoint theorem).[26] *Let $\mathfrak{F}(\mathfrak{x})$ be a completely continuous representation defined in the convex bounded set $V$.*[21] *If the image $\mathfrak{F}(S)$ of the boundary $S$ of $V$ lies in $V$, then there exists at least one fixpoint in $V$.*

*Proof.* Let us assume that there is no fixpoint on $S$, and, under this assumption, prove the existence of a fixpoint in the interior of $V$, that is, the existence of an interior solution of the equation $\mathfrak{x} = \mathfrak{F}(\mathfrak{x})$, or, what is the same, of the equation

$$\mathfrak{x} + \mathfrak{y}_0 - \mathfrak{F}(\mathfrak{x}) = \mathfrak{y}_0 \tag{2.19}$$

in which $\mathfrak{y}_0$ is an arbitrary but fixed interior point of V. Since $u(\mathfrak{x}, S, \mathfrak{y}_0) = 1$, it follows from Theorem 8 that for the proof of (2.19) it will be sufficient to show that, with $\mathfrak{g}(\mathfrak{x}) = \mathfrak{x} + \mathfrak{y}_0 - \mathfrak{F}(\mathfrak{x})$,

$$u(\mathfrak{g}, S, \mathfrak{y}_0) = u(\mathfrak{x}, S, \mathfrak{y}_0). \tag{2.20}$$

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[25] Since $\mathfrak{f}_0(\mathfrak{x}) = \mathfrak{x} + [(1-t)(\mathfrak{y}_1 - \mathfrak{x}_1) + t\overline{\mathfrak{F}_0}(\mathfrak{x})]$ where $\overline{\mathfrak{F}_0}(\mathfrak{x}) = \mathfrak{F}_0(\bar{\mathfrak{x}})$ for $\mathfrak{x} + \mathfrak{x}_1$ and $= 0$ for $\mathfrak{x} = \mathfrak{x}_1$ it will be clear that $\mathfrak{f}_0$ is a representation (1.1) when it is proved that $t$ and $t\mathfrak{F}_0(\bar{\mathfrak{x}})$ depend continuously on $\mathfrak{x}$. This continuity is obvious for $\mathfrak{x} = \mathfrak{x}_1$. For $\mathfrak{x} + \mathfrak{x}_1$, see the proof of Lemma 9, Section 4.

[26] For literature concerning fixpoint theorems in function spaces see, for instance, [10], Footnote 12.

As is seen from the theorem of Poincaré-Bohl (Theorem 3), (2.20) will be proved if we can show that the equation

$$\mathfrak{x} + t[\mathfrak{y}_0 - \mathfrak{F}(\mathfrak{x})] = \mathfrak{y}_0 \tag{2.21}$$

has for no $t$ of the interval $0 \leqq t \leqq 1$ a solution $\mathfrak{x}$ which lies on $S$.

Since $\mathfrak{y}_0$ is an interior point of $V$, this is obvious for $t = 0$, and since, by assumption, (2.19) has no solution on $S$, it is also obvious for $t = 1$. Let us now assume that for a certain $t$-value of the open interval $0 < t < 1$ (2.21) has a solution $\mathfrak{x} \subset S$. Since, then,

$$\mathfrak{F}(\mathfrak{x}) = \mathfrak{y}_0 + \frac{\mathfrak{x} - \mathfrak{y}_0}{t} \qquad (\mathfrak{x} \subset S)$$

the image $\mathfrak{F}(\mathfrak{x})$ of $\mathfrak{x}$ would be in the exterior of $V$, which is in contradiction with the hypothesis of our theorem that $\mathfrak{F}(\mathfrak{x}) \subset V$ for $\mathfrak{x} \subset S$.

### 3. GENERAL SPACES

In this section, we denote by $E$ a space having the properties 1, 2, and 3 stated at the beginning of the introduction. The aim of the present section is to extend the notion of the order and the main theorems proved in Section 1 to such a space $E$.

According to a theorem proved by Kolmogoroff,[27] it is possible to introduce in $E$ a norm $||\mathfrak{x}||$ in such a manner that a point $\mathfrak{a} \subset E$ belongs then and only then to the closure $\overline{A}$ of the set $A \subset E$ if for each positive number $\varepsilon$ the full sphere $||\mathfrak{x} - \mathfrak{a}|| \leqq \varepsilon$ contains a point of $A$.[28] For the sake of brevity, we will call such a norm a consistent norm.

Now let $V$ be a convex bounded set in $E$ containing at least one interior point and $S$ its boundary. Let $\mathfrak{f}(\mathfrak{x})$ be a representation (1.1) with $\lambda \equiv 1$ defined on $S$. Let $||\mathfrak{x}||$ be a consistent norm introduced in $E$. Using this norm, we can, according to Section 1, define the order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ for any point $\mathfrak{y}_0 \subset E$ not lying on $\mathfrak{f}(S)$. Then the following lemma holds:

LEMMA 5. *The order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ is the same for all consistent norms.*

This lemma, the proof of which will be given afterwards, justifies the following:

DEFINITION 6. *Let $\mathfrak{f}(\mathfrak{x})$ be a representation* (1.1) *with $\lambda \equiv 1$ defined on the boundary $S$ of the convex bounded set $V \subset E$ which contains at least one interior point. The order $u(\mathfrak{f}, S, \mathfrak{y}_0)$ of the point $\mathfrak{y}_0$ not lying on $\mathfrak{f}(S)$ with respect to $\mathfrak{f}(S)$ is, by definition, equal to the order obtained by norming $E$ with a consistent norm. For a general representation* (1.1) *the order is then given by Definition* 3.

An immediate consequence of this definition and Lemma 5 is

[27] [5].

[28] In other words, the topological correspondence given by defining $||\mathfrak{x} - \mathfrak{y}||$ as the distance between $\mathfrak{x}$ and $\mathfrak{y}$ is the same as the original correspondence between $\overline{A}$ and $A$ (see, for instance, [2], p. 28).

THEOREM 11. *The following theorems of Section* 1 *hold for any space* $E$ *satisfying Conditions* 1, 2, *and* 3 *of the introduction: Theorems* 1, 2, 3, 6, 7, 8, *and* 10.

*Proof of Lemma* 5. For any point $\mathfrak{x} \subset E$, we denote by $||\mathfrak{x}||_i$ $(i = 1, 2)$ two consistent norms of $\mathfrak{x}$, and by $V_i^{\mathfrak{y}}$ a point set which is, with respect to the corresponding norm, a full sphere with center $\mathfrak{y}$. Since $\mathfrak{y}_0$ does not lie on $\mathfrak{f}(S)$, there exists a positive number $\varepsilon_1$ such that the $V_1^{\mathfrak{y}}$ with radius $\varepsilon_1$ contains no point of $\mathfrak{f}(S)$. Since $||\mathfrak{x}||_1$ and $||\mathfrak{x}||_2$ are both consistent norms, the systems of neighborhoods formed by the full spheres with respect to the two norms are topologically equivalent.[29] Hence, there exists a $V_2^{\mathfrak{y}_0}$ with radius $\varepsilon_2 > 0$ such that

$$V_2^{\mathfrak{y}_0} \subset V_1^{\mathfrak{y}_0} \tag{3.1}$$

Now let $\mathfrak{s}$ be a layer representation for which

$$||\mathfrak{f}(\mathfrak{x}) - \mathfrak{s}(\mathfrak{x})||_2 < \varepsilon_2. \tag{3.2}$$

We state that (3.2) implies

$$||\mathfrak{f}(\mathfrak{x}) - \mathfrak{s}(\mathfrak{x})||_1 < \varepsilon_1. \tag{3.3}$$

To show this, we remark that (3.2) and (3.3) are equivalent with

$$\mathfrak{s}(\mathfrak{x}) \subset V_2^{\mathfrak{y}} \tag{3.2a}$$

and

$$\mathfrak{s}(\mathfrak{x}) \subset V_1^{\mathfrak{y}} \tag{3.3a}$$

respectively, if $\mathfrak{y} = \mathfrak{f}(\mathfrak{x})$ and $\varepsilon_i$ is the radius of $V_i^{\mathfrak{y}}$. But since the translation $\mathfrak{x}' = \mathfrak{x} + \mathfrak{y} - \mathfrak{y}_0$ transforms $V_i^{\mathfrak{y}_0}$ into $V_i^{\mathfrak{y}}$ $(i = 1, 2)$, (3.1) implies $V_2^{\mathfrak{y}} \subset V_1^{\mathfrak{y}}$. Hence, (3.3a) is indeed a consequence of (3.2a).

Let us now denote by $E_i$ the space $E$ if normed with the norm $||\mathfrak{x}||_i$ and by $u_i$ the order in the normed space $E_i$. With these notations, it follows from (3.2, (3.3), and Definition 2 in Section 2 that

$$u_1(\mathfrak{f}, S, \mathfrak{y}_0) = u_1(\mathfrak{s}, S, \mathfrak{y}_0), \quad u_2(\mathfrak{f}, S, \mathfrak{y}_0) = u_2(\mathfrak{s}, S, \mathfrak{y}_0). \tag{3.4}$$

We now consider an $n$-dimensional linear subspace $E^n$ of $E$ which satisfies the conditions (a), (b), and (c) of Definition 1, Section 2. With the notations used in this definition, it is a well known fact in the topology of $n$-dimensional spaces that the order $u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0)$ of $\mathfrak{y}_0$ with respect to the image $\mathfrak{s}^n(S^{n-1})$ (which is in $E^n$) is the same whether we consider $E^n$ as normed by the norm $||\mathfrak{x}||_1$ or by the norm $||\mathfrak{x}||_2$. Therefore, it follows from Definition 1 that

$$u_1(\mathfrak{s}, S, \mathfrak{y}_0) = u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0), \quad u_2(\mathfrak{s}, S, \mathfrak{y}_0) = u(\mathfrak{s}^n, S^{n-1}, \mathfrak{y}_0).$$

Hence, on account of (3.4): $u_1(\mathfrak{f}, S, \mathfrak{y}_0) = u_2(\mathfrak{f}, S, \mathfrak{y}_0)$.

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[29] See, for instance, [2], p. 31 ("Hausdorffsches Gleichwertigkeitskriterium").

## 4. STRICTLY CONVEX SPACES

According to the theorem of Kolmogoroff mentioned in Section 3,[30] it is possible to introduce in $E$ a consistent norm. Now we make the assumption that it is possible to introduce such a consistent norm that the space $E$ after being normed is strictly convex.[31] In the present section we will always assume that such a norm has been introduced, and the normed space will again be denoted by $E$.

Using our usual notations, let $V$ be a convex bounded set containing at least one interior point, $S$ its boundary, and

$$\mathfrak{f}_1(\mathfrak{x}) = \lambda_1(\mathfrak{x})\,\mathfrak{x} + \mathfrak{F}_1(\mathfrak{x}), \quad \mathfrak{f}_2(\mathfrak{x}) = \lambda_2(\mathfrak{x})\,\mathfrak{x} + \mathfrak{F}_2(\mathfrak{x}) \tag{4.1}$$

two representations (1.1) defined on $S$. We then prove

THEOREM 12. *Let $\mathfrak{y}_0$ be a point lying on neither $\mathfrak{f}_1(S)$ nor $\mathfrak{f}_2(S)$. The necessary and sufficient condition that $\mathfrak{f}_1$ and $\mathfrak{f}_2$ be homotopic in $E - \mathfrak{y}_0$[32] is that*

$$u(\mathfrak{f}_1, S, \mathfrak{y}_0) = u(\mathfrak{f}_2, S, \mathfrak{y}_0) \tag{4.2}$$

As a preparation for the proof of Theorem 12, we prove the following lemmas.

LEMMA 6. *Let $E^n$ be an $n$-dimensional linear subset of $E$. To each $\mathfrak{x} \subset E$ corresponds one and only one $\bar{\mathfrak{x}} \subset E^n$ such that for all points $\mathfrak{x}_n \subset E^n$ the inequality*

$$||\mathfrak{x} - \bar{\mathfrak{x}}|| \leqq ||\mathfrak{x} - \mathfrak{x}_n|| \tag{4.3}$$

*holds.*[33]

*Proof.* From the inequalities $|\,||\mathfrak{x} - \mathfrak{x}_n'|| - ||\mathfrak{x} - \mathfrak{x}_n''||\,| \leqq ||\mathfrak{x}_n' - \mathfrak{x}_n''||$ and $||\mathfrak{x}_n - \mathfrak{x}|| \geqq ||\mathfrak{x}_n|| - ||\mathfrak{x}||$ it follows immediately that $||\mathfrak{x} - \mathfrak{x}_n||$ is a continuous function of $\mathfrak{x}_n$ and that $\lim_{||\mathfrak{x}_n|| \to \infty} ||\mathfrak{x} - \mathfrak{x}_n|| = \infty$. Hence, $||\mathfrak{x} - \mathfrak{x}_n||$ has a minimum, that is, there exists at least one point $\mathfrak{x}_n = \bar{\mathfrak{x}} \subset E^n$ for which (4.3) holds. It remains to be proved that there exists only one such point $\bar{\mathfrak{x}}$. This is obvious if $\mathfrak{x} \subset E^n$ since then, obviously, $\bar{\mathfrak{x}} = \mathfrak{x}$. Let us now assume that $\mathfrak{x}$ is not in $E^n$. If then $\bar{\mathfrak{x}}$ is a point satisfying (4.3), and $E^{n+1}$ the $n+1$-dimensional subspace of $E$ which contains $E^n$ and $\mathfrak{x}$, we construct in $E^{n+1}$ the sphere $S^n$ with center $\mathfrak{x}$ and radius $||\mathfrak{x} - \bar{\mathfrak{x}}||$. On account of (4.3), $E^n$ is then a plane of support of $S^n$. But since $E^{n+1}$ is strictly convex, $S^n$

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[30] See Footnote 27.

[31] According to a theorem of Clarkson [4], this assumption is, for instance, fulfilled for any separable Banach space. For the definition of a strictly convex space, see Footnote 11.

[32] Cf. Definition 4, Section 2.

[33] The following example shows that Lemma 6 is not true in a space which is not strictly convex: Let $E$ be a two-dimensional Euclidean plane with rectangular co-ordinates $x$, $y$ and let $|x| + |y|$ be the norm of the point of co-ordinates $(x, y)$. Let $E^n = E'$ be the straight line $x + y - 1 = 0$, and $\mathfrak{x}$ the point $x = 0$, $y = 0$. The inequality (4.3) is then satisfied for all points $\bar{\mathfrak{x}}$ of $E'$ for which both co-ordinates are non-negative.

has, according to a theorem of Minkowski,[34] exactly one point in common with any supporting plane. For the supporting plane $E^n$ this point is $\bar{\mathfrak{x}}$ which, therefore, is uniquely determined.

Lemma 6 justifies the following:

DEFINITION 7. *The point $\bar{\mathfrak{x}}$ uniquely defined by the inequality (4.3) is called the projection of $\mathfrak{x}$ on $E^n$. $d(\mathfrak{x}) = ||\mathfrak{x} - \bar{\mathfrak{x}}||$ is called the distance between $\mathfrak{x}$ and $E^n$.*

LEMMA 7. *The distance $d(\mathfrak{x})$ between $\mathfrak{x}$ and its projection $\bar{\mathfrak{x}}$ on $E^n$ is continuous.*

*Proof.* Let $\mathfrak{x}$, $\mathfrak{x}_0$ be two points of $E$, and $\bar{\mathfrak{x}}$, $\bar{\mathfrak{x}}_0$ their projections on $E^n$. We get from (4.3) with $\mathfrak{x}_n = \bar{\mathfrak{x}}_0$

$$d(\mathfrak{x}) \leqq ||\mathfrak{x} - \bar{\mathfrak{x}}_0|| \leqq ||\mathfrak{x} - \mathfrak{x}_0|| + ||\mathfrak{x}_0 - \bar{\mathfrak{x}}_0|| = ||\mathfrak{x} - \mathfrak{x}_0|| + d(\mathfrak{x}_0).$$

Since the inequality obtained from the preceding one by interchanging $\mathfrak{x}$ and $\mathfrak{x}_0$ also holds, we have $d(\mathfrak{x}_0) - ||\mathfrak{x} - \mathfrak{x}_0|| \leqq d(\mathfrak{x}) \leqq d(\mathfrak{x}_0) + ||\mathfrak{x} - \mathfrak{x}_0||$, which proves the continuity of $d(\mathfrak{x})$.

LEMMA 8. *The projection $\bar{\mathfrak{x}}$ of $\mathfrak{x}$ on $E^n$ depends continuously on $\mathfrak{x}$.*

*Proof.* Let $\mathfrak{x}_1, \mathfrak{x}_2, \ldots$ be a sequence with $\lim_{\nu\to\infty} \mathfrak{x}_\nu = \mathfrak{x}$; let $\bar{\mathfrak{x}}_\nu$ be the projections of $\mathfrak{x}_\nu$ on $E^n$, $d = ||\mathfrak{x} - \bar{\mathfrak{x}}||$, and $d_\nu = ||\mathfrak{x}_\nu - \bar{\mathfrak{x}}_\nu||$. It is to be proved that

$$\lim_{\nu\to\infty} \bar{\mathfrak{x}}_\nu = \bar{\mathfrak{x}}. \tag{4.4}$$

Since, on account of Lemma 7, $\lim_{\nu\to\infty} d_\nu = d$, it follows from the inequalities

$$||\bar{\mathfrak{x}}_\nu - \bar{\mathfrak{x}}|| \leqq ||\bar{\mathfrak{x}}_\nu - \mathfrak{x}_\nu|| + ||\mathfrak{x}_\nu - \mathfrak{x}|| + ||\mathfrak{x} - \bar{\mathfrak{x}}|| = d_\nu + ||\mathfrak{x}_\nu - \mathfrak{x}|| + d$$

that the set of all $\bar{\mathfrak{x}}_\nu$ is a bounded subset of $E^n$. Therefore, the set $\bar{\mathfrak{x}}_\nu$ is compact and for the proof of (4.4) it is sufficient to show that (4.4) holds for any subsequence $n_\nu$ for which $\bar{\mathfrak{x}}_{n_\nu}$ converges. If then $\mathfrak{x}' = \lim_{\nu\to\infty} \bar{\mathfrak{x}}_{n_\nu}$, we have

$$||\mathfrak{x} - \mathfrak{x}'|| \leqq ||\mathfrak{x} - \mathfrak{x}_{n_\nu}|| + ||\mathfrak{x}_{n_\nu} - \bar{\mathfrak{x}}_{n_\nu}|| + ||\bar{\mathfrak{x}}_{n_\nu} - \mathfrak{x}'||.$$

Since the first and last terms converge to zero, and, on account of Lemma 7, the second term to $d$, it follows $||\mathfrak{x} - \mathfrak{x}'|| \leqq d$ which inequality, according to the definition of $\bar{\mathfrak{x}}$ and to Lemma 6, implies $\mathfrak{x}' = \bar{\mathfrak{x}}$, as we wished to prove.

We turn now to the proof of Theorem 12. In view of Theorem 2, Section 2, only the sufficiency of (4.2) has to be proved. If we put

$$\bar{\mathfrak{f}}_i(\mathfrak{x}) = \mathfrak{y}_0 + \frac{1}{\lambda_i(\mathfrak{x})}[\mathfrak{f}_i(\mathfrak{x}) - \mathfrak{y}_0] \qquad (i = 1, 2)$$

we see from Definition 3, Section 2, and the hypothesis (4.2) that

$$u(\bar{\mathfrak{f}}_1, S, \mathfrak{y}_0) = u(\bar{\mathfrak{f}}_2, S, \mathfrak{y}_0). \tag{4.5}$$

Putting

$$\lambda_i(\mathfrak{x}, t) = 1 + t[\lambda_i(\mathfrak{x}) - 1] \qquad (i = 1, 2,\ 0 \leqq t \leqq 1)$$

[34] [8], pp. 38, 39.

one sees easily that $\mathfrak{f}_1$ and $\bar{\mathfrak{f}}_1$ are homotopic in $E - \mathfrak{y}_0$ as are likewise $\mathfrak{f}_2$ and $\bar{\mathfrak{f}}_2$. It is therefore sufficient to prove that $\bar{\mathfrak{f}}_1$ and $\bar{\mathfrak{f}}_2$ are homotopic in $E - \mathfrak{y}_0$.

Now let $\varepsilon_1$ be the distance between $\mathfrak{y}_0$ and $\mathfrak{f}_1(S)$, and $\varepsilon$ a positive number smaller than $\frac{\varepsilon_1}{M}$ and $\frac{\varepsilon_2}{M}$. If then $\mathfrak{s}_1(\mathfrak{x}) = \mathfrak{x} + \mathfrak{S}_1(\mathfrak{x})$ are layer representations with $||\bar{\mathfrak{f}}_1(\mathfrak{x}) - \mathfrak{s}_1(\mathfrak{x})|| < \varepsilon$, we have, on account of (4.5) and Definition 2,

$$u(\mathfrak{s}_1, S, \mathfrak{y}_0) = u(\mathfrak{s}_2, S, \mathfrak{y}_0) \tag{4.6}$$

Putting

$$\bar{\mathfrak{f}}_1(\mathfrak{x}, t) = (1-t)\bar{\mathfrak{f}}_1(\mathfrak{x}) + t\mathfrak{s}_1(\mathfrak{x}),$$

one sees easily that $\mathfrak{f}_1$ and $\mathfrak{s}_1$ are homotopic in $E - \mathfrak{y}_0$, and likewise $\bar{\mathfrak{f}}_2$ and $\mathfrak{s}_2$.

It remains to be proved that $\mathfrak{s}_1$ and $\mathfrak{s}_2$ are homotopic in $E - \mathfrak{y}_0$. To show this, let $E^n$ be a linear subspace of $E$ having the properties (a), (b), and (c) given in Definition 1 (Section 2) with respect to $\mathfrak{s}_1$ as well as to $\mathfrak{s}_2$. Let $S^{n-1}$ denote the intersection of $S$ and $E^n$, and $\mathfrak{s}_i^n(\mathfrak{x}) = \mathfrak{x} + \mathfrak{S}_i^n(\mathfrak{x}) = \mathfrak{s}_i(\mathfrak{x})$ for $\mathfrak{x} \subset S^{n-1}$ $(i = 1, 2)$. We have then from Definition 1 in connection with (4.6)

$$u(\mathfrak{s}_1^n, S, \mathfrak{y}_0) = u(\mathfrak{s}_2^n, S, \mathfrak{y}_0).$$

Now, it is well known from the topology of $n$-dimensional spaces that this equality is sufficient (and necessary) for the homotopy of $\mathfrak{s}_1^n$ and $\mathfrak{s}_2^n$ in $E^n - \mathfrak{y}_0$.[35] Therefore, there exists a representation $\mathfrak{s}^n(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}^n(\mathfrak{x}, t)$ defined for $\mathfrak{x} \subset S^{n-1}$ and for $0 \leqq t \leqq 1$, continuous in $(\mathfrak{x}, t)$, such that

$$\mathfrak{s}^n(\mathfrak{x}, 0) = \mathfrak{s}_1^n(\mathfrak{x}), \quad \mathfrak{s}^n(\mathfrak{x}, 1) = \mathfrak{s}_2^n(\mathfrak{x}), \quad \mathfrak{s}^n(\mathfrak{x}, t) + \mathfrak{y}_0. \tag{4.7}$$

We now define a representation for all $\mathfrak{x} \subset E^n$ which for $\mathfrak{x} \subset S^{n-1}$ is identical with $\mathfrak{s}^n(\mathfrak{x}, t)$ and denote this extended representation again by $\mathfrak{s}^n(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}^n(\mathfrak{x}, t)$.[36] Let then $\mathfrak{x}$ be a point on $S$, $\bar{\mathfrak{x}}$ its projection on $E^n$ (Definition 7), and $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{x} + \mathfrak{S}(\mathfrak{x}, t)$ the representation defined on $S$ and given by

$$\begin{aligned}\mathfrak{S}(\mathfrak{x}, t) = {} & \mathfrak{S}^n(\bar{\mathfrak{x}}, t) + \mathfrak{S}_1(\mathfrak{x}) - \mathfrak{S}^n(\bar{\mathfrak{x}}, 0) \\ & + t\{[\mathfrak{S}_2(\mathfrak{x}) - \mathfrak{S}^n(\bar{\mathfrak{x}}, 1)] - [\mathfrak{S}_1(\mathfrak{x}) - \mathfrak{S}^n(\bar{\mathfrak{x}}, 0)]\}\end{aligned} \tag{4.8}$$

In order to prove the homotopy of $\mathfrak{s}_1$ and $\mathfrak{s}_2$ in $E - \mathfrak{y}_0$, we will show that $\mathfrak{s}(\mathfrak{x}, t)$ possesses the properties required in Definition 4, Section 2. First we have, clearly, $\mathfrak{s}(\mathfrak{x}, 0) = \mathfrak{s}_1(\mathfrak{x})$ and $\mathfrak{s}(\mathfrak{x}, 1) = \mathfrak{s}_2(\mathfrak{x})$. Moreover, it

[35] See, for instance, [2], p. 511, Satz I', in connection with p. 462, Section 5.

[36] Such a representation may, for instance, be obtained in the following manner: let $V^n$ be the intersection of $V$ and $E^n$ so that $S^{n-1}$ is the boundary of $V^n$. Let $\mathfrak{x}_0$ be an arbitrary but fixed interior point of $V^n$. By definition we put $\mathfrak{s}^n(\mathfrak{x}_0, t) = \mathfrak{y}_0$. For any other point $\mathfrak{x} \subset E$ let $\bar{\mathfrak{x}}$ be the uniquely determined (cf. Footnote 39) intersection of $S^{n-1}$ with the ray issuing from $\mathfrak{x}_0$ and containing $\mathfrak{x}$. If then $\mathfrak{x} = \mathfrak{x}_0 + \tau(\bar{\mathfrak{x}} - \mathfrak{x}_0)$, we put $\mathfrak{s}^n(\mathfrak{x}, t) = \mathfrak{y}_0 + \tau[\mathfrak{s}^n(\bar{\mathfrak{x}}, t) - \mathfrak{y}_0]$.

is immediately seen from (4.8) and Lemma 8 that $\mathfrak{S}$ is continuous. Furthermore, the set of all points $\mathfrak{S}(\mathfrak{x}, t)$ $(\mathfrak{x} \subset S,\ 0 \leqq t \leqq 1)$ is compact since this set is a bounded set of $E^n$.[37] Finally, for $\mathfrak{x} \subset S$, $\mathfrak{y}_0$ is different from $\mathfrak{s}(\mathfrak{x}, t)$. In fact, since $\mathfrak{y}_0$ and $\mathfrak{S}(\mathfrak{x}, t)$ are both points of $E^n$, this is obvious for a point $\mathfrak{x}$ which does not lie in $E^n$. But if $\mathfrak{x} \subset E^n$, then $\mathfrak{x} = \bar{\mathfrak{x}}$ and, according to (4.7) and (4.8), $\mathfrak{s}(\mathfrak{x}, t) = \mathfrak{s}^n(\mathfrak{x}, t)$; but $\mathfrak{s}^n(\mathfrak{x}, t)$ is [cf. (4.7)] different from $\mathfrak{y}_0$ since $\mathfrak{x} \subset S^{n-1}$.

We turn now to the notion of the degree $\gamma(\mathfrak{f}, S)$ of a representation (1.1) which is defined on the boundary $S$ of a convex bounded set $V \subset E$ and for which the image $\mathfrak{f}(S)$ lies on the boundary $S_1$ of a convex bounded set $V_1 \subset E$; each of the sets $V$ and $V_1$ is supposed to contain at least one interior point. The order $u(\mathfrak{f}, \mathfrak{S}, \mathfrak{y}_0)$ is, according to Theorem 1, Section 2, the same for all interior points $\mathfrak{y}_0$ of $V_1$. This justifies the following

DEFINITION 8. *The degree* $\gamma(\mathfrak{f}, S)$ *is defined by the equation*

$$\gamma(\mathfrak{f}, S) = u(\mathfrak{f}, S, \mathfrak{y}_0)$$

*where* $\mathfrak{y}_0$ *is an arbitrary interior point of* $V_1$.[38]

THEOREM 13. *If* $S_1$ *contains a point* $\mathfrak{y}_1$ *which is not an image point under the representation* (1.1) *mapping* $S$ *on* $S_1$, *that is, for which* $\mathfrak{f}(\mathfrak{x}) \neq \mathfrak{y}_1$ *for all* $\mathfrak{x} \subset S$, *then* $\gamma(\mathfrak{f}, S) = 0$.

The proof follows immediately from Theorem 7, Section 2, if one takes as the ray $h$ of this theorem the ray issuing from $\mathfrak{y}_0$ (Definition 8) and containing the point $\mathfrak{y}_1$ of Theorem 13.[39]

Let $\mathfrak{f}_1$ and $\mathfrak{f}_2$ be two representations (1.1) mapping $S$ on $S_1$. If there exists a representation $\mathfrak{f}(\mathfrak{x}, t)$ satisfying the conditions of Definition 4, Section 2, and for which, moreover, $\mathfrak{f}(\mathfrak{x}, t) \subset S_1$ for $\mathfrak{x} \subset S$ and for $0 \leqq t \leqq 1$, we say that $\mathfrak{f}_1$ and $\mathfrak{f}_2$ are homotopic in $S_1$. With these notations, we prove

THEOREM 14. *The equality*

$$\gamma(\mathfrak{f}_1, S) = \gamma(\mathfrak{f}_2, S) \tag{4.9}$$

*is a neccessary and sufficient condition for the homotopy in* $S_1$ *of* $\mathfrak{f}_1$ *and* $\mathfrak{f}_2$.[40]

Before proving Theorem 14 we state the following

LEMMA 9. *Let* $\mathfrak{y}_0$ *be an interior point of the convex bounded set* $V_1 \subset E$. *For any point* $\mathfrak{y} \subset E$ *different from* $\mathfrak{y}_0$ *let* $\bar{\mathfrak{y}}$ *be the uniquely determined*[39]

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[37] That $\mathfrak{S}(\mathfrak{x}, t) \subset E^n$ follows from (4.8) and from the fact that $\mathfrak{s}_1(\mathfrak{x}, t)$ and $\mathfrak{s}_2(\mathfrak{x}, t)$ are layer representations with respect to $E^n$. That the set of all $\mathfrak{S}(\mathfrak{x}, t)$ is bounded is a consequence of (4.8) and the fact that the set of all projections $\bar{\mathfrak{x}}$ of the bounded set $S$ is bounded, as is easily seen from the inequality $||\bar{\mathfrak{x}} - \mathfrak{x}_0|| \leqq ||\bar{\mathfrak{x}} - \mathfrak{x}|| + ||\mathfrak{x} - \mathfrak{x}_0|| \leqq 2||\mathfrak{x} - \mathfrak{x}_0||$ where $\mathfrak{x}_0$ is the point defined in the preceding footnote.

[38] Definition 8 is in agreement with the notations used in the topology of $n$-dimensional spaces.

[39] For a proof that a ray issuing from an interior point $\mathfrak{y}_0$ of $V_1$ intersects the boundary $S_1$ of $V_1$ in exactly one point see the proof given in [2], p. 599, for the $n$-dimensional case; this proof, however, is, wthout any change, valid also in the case of the space $E$.

[40] In the case that $E$ is the Hilbert space and $S$ identical with $S_1$ this theorem has been proved in [9].

*intersection of the ray issuing from* $\mathfrak{y}_0$ *and containing* $\mathfrak{y}$ *and of the boundary* $S_1$ *of* $V_1$. *Then* $\bar{\mathfrak{y}}$ *depends continuously on* $\mathfrak{y}$.[41]

*Proof of Lemma* 9. For $\mathfrak{z} \subset E$, let $t(\mathfrak{z})$ be the real function defined by

$$t(\mathfrak{z}) = \frac{||\mathfrak{z}||}{||(\overline{\mathfrak{y}_0 + \mathfrak{z}}) - \mathfrak{y}_0||} \qquad (\mathfrak{z} \neq 0). \tag{4.10}$$

We have then

$$\bar{\mathfrak{y}} = \mathfrak{y}_0 + \frac{1}{t(\mathfrak{y} - \mathfrak{y}_0)}(\mathfrak{y} - \mathfrak{y}_0). \tag{4.11}$$

In order to prove that $\bar{\mathfrak{y}}$ is continuous in dependence on $\mathfrak{y}$ for $\mathfrak{y} \neq \mathfrak{y}_0$, it will be sufficient to prove that $t(\mathfrak{y} - \mathfrak{y}_0)$ is continuous as function of $\mathfrak{y}$ since, according to (4.10),

$$t(\mathfrak{y} - \mathfrak{y}_0) > 0 \qquad \text{for } \mathfrak{y} \neq \mathfrak{y}_0. \tag{4.12}$$

To prove this continuity of $t$ it will evidently be sufficient to prove that for any two points $\mathfrak{y}_1 \neq \mathfrak{y}_2$ different from $\mathfrak{y}_0$ the inequality

$$t(\mathfrak{y}_2 - \mathfrak{y}_0) \leqq t(\mathfrak{y}_2 - \mathfrak{y}_1) + t(\mathfrak{y}_1 - \mathfrak{y}_0) \tag{4.13}$$

holds since then, on account of (4.10),

$$|t(\mathfrak{y}_2 - \mathfrak{y}_0) - t(\mathfrak{y}_1 - \mathfrak{y}_0)| \leqq t(\mathfrak{y}_2 - \mathfrak{y}_1) \leqq \frac{||\mathfrak{y}_1 - \mathfrak{y}_2||}{\varepsilon}$$

where $\varepsilon$ is so small that the full sphere with center $\mathfrak{y}_0$ and radius $\varepsilon$ lies in the interior of $V_1$.

To prove (4.13) we first note that for any positive real number $\alpha$

$$t[\alpha(\mathfrak{y} - \mathfrak{y}_0)] = \alpha t(\mathfrak{y} - \mathfrak{y}_0) \tag{4.14}$$

since, obviously, $\overline{[\mathfrak{y}_0 + (\mathfrak{y} - \mathfrak{y}_0)]} = \overline{[\mathfrak{y}_0 + \alpha(\mathfrak{y} - \mathfrak{y}_0)]} = \bar{\mathfrak{y}}$.

Let us now assume that (4.13) does not hold for a certain pair of points $\mathfrak{y}_1 \neq \mathfrak{y}_2$ different from $\mathfrak{y}_0$.[42] This assumption would imply the existence of a number $\tau$ for which

$$t(\mathfrak{y}_2 - \mathfrak{y}_0) > \tau > t(\mathfrak{y}_2 - \mathfrak{y}_1) + t(\mathfrak{y}_1 - \mathfrak{y}_0). \tag{4.15}$$

We consider then the points $\mathfrak{y}_3$, $\mathfrak{y}_4$, $\mathfrak{y}_5$, and $\mathfrak{y}_6$ defined by

$$\mathfrak{y}_3 = \mathfrak{y}_0 + \frac{1}{\tau}(\mathfrak{y}_1 - \mathfrak{y}_0) \qquad \mathfrak{y}_4 = \mathfrak{y}_3 + \frac{1}{\tau}(\mathfrak{y}_2 - \mathfrak{y}_1)$$

$$\mathfrak{y}_5 = \mathfrak{y}_0 + \frac{(\mathfrak{y}_3 - \mathfrak{y}_0)}{1 - \theta} \qquad \mathfrak{y}_6 = \mathfrak{y}_0 + \frac{\mathfrak{y}_4 - \mathfrak{y}_3}{\theta},$$

where

$$\theta = \frac{t(\mathfrak{y}_4 - \mathfrak{y}_3)}{t(\mathfrak{y}_3 - \mathfrak{y}_0) + t(\mathfrak{y}_4 - \mathfrak{y}_3)}.$$

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[41] Lemma 9 holds in any space $E$ as defined in Section 1.

[42] The ensuing part of the proof follows Minkowski [8], pp. 12, 13, and is reproduced for the convenience of the reader.

From these definitions in connection with (4.15), (4.14), and (4.12) it follows easily that

$$t(\mathfrak{y}_3 - \mathfrak{y}_0) = \frac{1}{\tau} t(\mathfrak{y}_1 - \mathfrak{y}_0) < 1, \qquad t(\mathfrak{y}_4 - \mathfrak{y}_0) = \frac{1}{\tau} t(\mathfrak{y}_2 - \mathfrak{y}_0) > 1,$$

$$t(\mathfrak{y}_5 - \mathfrak{y}_0) = t(\mathfrak{y}_6 - \mathfrak{y}_0) = t(\mathfrak{y}_3 - \mathfrak{y}_0) + t(\mathfrak{y}_4 - \mathfrak{y}_3)$$

$$= \frac{1}{\tau} [t(\mathfrak{y}_1 - \mathfrak{y}_0) + t(\mathfrak{y}_2 - \mathfrak{y}_1)] < 1.$$

Since evidently $t(\mathfrak{y} - \mathfrak{y}_0) < 1$ for an interior and $> 1$ for an exterior point $\mathfrak{y}$, these inequalities imply

$$\mathfrak{y}_5 \subset V_1, \mathfrak{y}_6 \subset V_1, \mathfrak{y}_4 \notin V_1. \tag{4.16}$$

Since $V_1$ is convex, (4.16) is in contradiction with the fact that $\mathfrak{y}_4 = (1 - \theta)\mathfrak{y}_5 + \theta\mathfrak{y}_6$ and $0 < \theta < 1$. Thus (4.13) and, therefore, Lemma 9 are proved.

*Proof of Theorem* 14. The necessity of the condition (4.8) is clear from Theorem 2, Section 2. To prove that (4.8) is also sufficient, let $\mathfrak{y}_0$ be an arbitrary but fixed interior point of $V_1$. According to Definition 8, and Theorem 12, $\mathfrak{f}_1$ and $\mathfrak{f}_2$ are then homotopic in $E - \mathfrak{y}_0$. Hence, there exists a representation $\mathfrak{f}(\mathfrak{x}, t)$ defined on $S$ for $0 \leqq t \leqq 1$ which is different from $\mathfrak{y}_0$ and satisfies the conditions of Definition 4, Section 2. If then $\bar{\mathfrak{y}}$ denotes the intersection of $S_1$ with the ray issuing from $\mathfrak{y}_0$ and containing the point $\mathfrak{y} = \mathfrak{f}(\mathfrak{x}, t)$, it is, on account of Lemma 9, easy to see that

$$\mathfrak{g}(\mathfrak{x}, t) = \frac{||\bar{\mathfrak{y}} - \mathfrak{y}_0||}{||\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0||} [\mathfrak{f}(\mathfrak{x}, t) - \mathfrak{y}_0]$$

is a representation (1.1) satisfying the conditions of Definition 4, Section 2, for which, moreover, $\mathfrak{g}(\mathfrak{x}, t)$ is always a point of $S_1$.

## REFERENCES


1. Alexandroff, P.
   1916. Über stetige Abbildungen kompakter Räume. Mathematische Annalen, 96:555-571.

2. Alexandroff and Hopf
   1935. Topologie, Erster Band. Julius Springer, Berlin.

3. Banach, S.
   1932. Théorie des opérations linéaires. Warszawa.

4. Clarkson, J. A.
   1936. Uniformly convex spaces. Transactions American Mathematical Society, 40:396-414.

5. Kolmogoroff
   1934. Zur Normierbarkeit allgemeiner linearer topologischer Räume. Studia Mathematica, 5:29.

6. Leray, J.
   1936. Les problèmes non linéaires. L'enseignement mathématique, 35:139-151.

7. LERAY and SCHAUDER
1934. Topologie et équations fonctionnelles. Annales Scientifiques de l'École Normale Supérieure, 51:45-78.

8. MINKOWSKI
1910. Geometrie der Zahlen. Leipzig and Berlin.

9. ROTHE, E.
1937. Über Abbildungsklassen von Kugeln des Hilbertschen Raumes. Compositio Mathematica, 4:294-307.

10. ———
1937. Zur Theorie der topologischen Ordnung und der Vektorfelder in Banachschen Räumen. Compositio Mathematica, 5:177-197.

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